UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

Office of Prevention, Pesticides
and
Toxic Substances

March 28, 2000

MEMORANDUM

SUBJECT: ***MALATHION:*** Evaluation of Hazard Identification Assessment Review Committee Reports Regarding Scientific Issues Presented by Dr. Brian Dementi

FROM: Virginia A. Dobozy, V.M.D., M.P.H.
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TO: Margaret Stasikowski, Director
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and

Jess Rowland, Co-Chair
Elizabeth Doyle, Co-Chair
Hazard Identification Assessment Review Committee
Health Effects Division

I've been asked by the Health Effects Division's (HED) senior management to respond to a February 28, 2000 letter from Dr. Brian Dementi, HED, to John Carley, Office of the Director, Office of Pesticide Programs, regarding the pesticide malathion. This letter summarized Dr. Dementi's perspective on seven "substance" (scientific) issues related to the assessment of non-cancer issues considered by HED's Hazard Identification Assessment Review Committee (HIARC). Each "substance" issue was summarized from existing letters/memoranda authored by Dr. Dementi and cited in the letter. The issues were submitted for consideration to an external peer review panel of toxicologists. Their comments/responses were evaluated by the HIARC in the December 22, 1998 Committee report. The letter also included eleven "process" issues, which are not addressed in this memorandum, although there is much overlap with the "substance" issues.

The purpose of this evaluation was to: 1) review the HIARC and FQPA Safety Factor Committee[1] reports to assure they are clear, accurate and transparent to an independent reader; and 2) review the same reports to assure Dr. Dementi's scientific opinions on the seven issues have been adequately considered, evaluated and documented by the HIARC. No position on the scientific issues was taken. Only the information provided in the committee reports, which included Dr. Dementi's detailed letters/memoranda as attachments, and other data cited in his February letter were reviewed. Appraisals of individual toxicology studies, such as Data Evaluation Records (DERs), were not reviewed. In the course of the evaluation, Dr. Dementi or others within OPP were contacted, as noted where applicable, for additional reports/information or clarification.

[1]Although the FQPA Safety Factor Committee report was not cited in Dr. Dementi's letter, it was considered in this evaluation because one of the issues related to the determination of the FQPA Safety Factor for malathion.

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Executive Summary

In a February 28, 2000 letter, Dr. Brian Dementi, HED, provided a summary of his scientific opinions on seven issues concerning the pesticide malathion. The issues are as follows:

1) Food Quality Protection Act (FQPA) 10X Safety Factor for Protection of Infants and Children
2) Hazard Identification/Acute Oral (One-Day)
3) Hazard Identification/Chronic Dietary (RfD)
4) Subchronic Inhalation Study
5) Acute Neurotoxicity Study (Retinal Histopathology)
6) Subchronic Neurotoxicity Study (Recommendation for Additional Behavioral Effects Testing)
7) Cholinesterase Inhibition - Enhanced Sensitivity of Females

Multiple questions for each issue and three general questions were submitted to an external peer review panel in 1998. The panel's responses/comments were discussed and evaluated by the Hazard Identification Assessment Review Committee (HIARC) on August 18, 20 and 27, 1998. The Committee's conclusions are presented in the December 22, 1998 HIARC report. [Prior to this, an internal (HED) peer review group examined three of the seven issues.] Dr. Dementi differs with the HIARC's conclusions on these issues, as stated in the summaries of his February letter and in numerous letters/memoranda addressed to the HIARC.

The purpose of this evaluation was to: 1) review the HIARC and FQPA Safety Factor Committee reports to assure they are clear, accurate and transparent to an independent reader; and 2) review the same reports to assure Dr. Dementi's scientific opinions on the seven issues have been adequately considered, evaluated and documented by the HIARC.
General comments on items 1 and 2 are provided. In addition, specific comments regarding the individual questions and issues are included under a detailed discussion of each issue.

In summary (evaluator's opinion):

1. Clarity, transparency and accuracy are lacking in the December 22, 1998 HIARC report regarding the discussion and deliberations on some of the peer review panel's responses/comments and the Committee's basis for its conclusions. In addition, there is no record in the report that three general questions submitted to the external peer review panel were discussed.

2. The December 17, 1997 and December 22, 1998 HIARC reports reflect that Dr. Dementi's scientific opinions were addressed and considered. Both internal (HED) and external peer review committees have addressed some or all of the seven issues.

3. The responses of the external peer review panel varied in their usefulness in sorting out the seven issues. The only issue on which there was uniform agreement was that there should be

follow-up on the retinal rosette findings (Issue 5). In many instances, the panel did not answer a question directly but instead referred to general toxicological principles. In some instances, their responses implied an misunderstanding of the question and/or EPA's risk assessment process. However, in their defense, some of the questions were complicated and possibly inappropriate for an external peer review panel. Dr. Dementi has listed what he thinks are the conclusions supported by *at least* (his emphasis) a consensus of the external reviewers at the end of Attachment 18 of his February 28, 2000 letter. This evaluator does not agree that these conclusions can be supported for the following reasons: 1) the panel offered individual opinions and did not express a collective or consensus opinion; 2) there was total agreement on very few of the responses; 3) many of the questions were not answered directly; and 4) Dr. Dementi misinterpreted some of the panel's responses.

Several of the issues are either partially or totally moot because they have been resolved by request of additional studies or EPA policy. A developmental neurotoxicity study is required under the September 10, 1999 Data Call-In (Issue 6). The registrant has agreed to conduct the study. Cholinesterase measurements in both adult and young organisms were added to the protocol, partially addressing Dr. Dementi's concerns under Issue 1. An inhalation study has also been required (Issue 4). Concerning Issue 3, Dr. Dementi's opinion is that the human study is the most appropriate study to use for endpoint selection for the chronic RfD. As EPA policy is currently in place that human studies will not be used for risk assessment, part of this issue is also moot.

Introduction

Two peer review panels, one internal and one external, have reviewed various scientific issues on which Dr. Dementi differed with the conclusions of the November 6, 1997 HIARC meeting. A summary of the panels' membership and their assignments is presented under Peer Review Process and HIARC's Evaluation of Panels' Responses/Comments. This evaluation provides both general and specific comments about the HIARC reports and whether Dr. Dementi's opinions have been addressed in these documents. The general comments appear first. Then, for each of the seven issues in Dr. Dementi's February 28, 2000 letter, the following approach is taken in this evaluation. Dr. Dementi's issue is presented verbatim from the February letter, along with his citations of detailed letters/memoranda.[2] (A complete list of the citations is in Attachment 1 of this document.) Information from these citations is summarized under Additional Information from Dr. Dementi's Detailed Memoranda/Letters. (There was one letter identified as Swetz99 in the attachments to the February letter which was not cited under any of the issues.) For each issue, the questions presented to the external peer review panel are summarized and followed by the responses of each panelist. (The actual questions are in Attachment 2 of this document.) Dr. Dementi's summary of the panel's responses is presented verbatim from Attachment 12, his consolidation report of the external peer review panel's responses. Additional comments from Dr. Dementi from this attachment are summarized or presented verbatim. Following this, the HIARC's summary of the panel's responses is taken verbatim from the December 22, 1998 HIARC report. After each question, this evaluator has expressed an opinion about the panel's responses [appears as In summary (evaluator's opinion)]. The HIARC report's conclusions about each question or issue are presented verbatim from the 1998 HIARC report. Finally, this evaluator offers personal opinions under Evaluator's Comments on Accuracy, Clarity and Transparency of HIARC Reports Concerning Issue x and Evaluator's Comments on HIARC Reports Reflecting Dr. Dementi's Opinions on Issue x.

Peer Review Process and HIARC's Evaluation of the Panels' Responses/Comments

After the November 6, 1997 HIARC meeting on malathion, both internal and external peer review mechanisms addressed multiple issues raised by Dr. Dementi on which he had a difference of scientific opinion with the HIARC. An internal (HED) ad hoc neurotoxicity subgroup was formed to consider and resolve three outstanding issues related to the neurotoxicity testing of malathion. All members of the subgroup were HED staff nominated by the branch chiefs of the two toxicology branches in existence at that time.[3] The subgroup met on November 13, 1997 to

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[2] In this document, some information is presented verbatim (italics), while some is summarized. Material was presented verbatim if: 1) it was thought to be critical to the issue; 2) was confusing and subject to misinterpretation; 3) couldn't be summarized adequately.

[3] This evaluator was a member of the ad hoc neurotoxicity subgroup. In consultation with John Carley, Office of Director, OPP, it was concluded that participation in the subgroup did not qualify as a

consider the following issues: 1) the possible greater sensitivity of females (as compared to males) to the cholinesterase inhibiting effects of malathion, and how this sex difference might affect the RfD for this chemical; 2) should EPA require the registrant to submit the microscopic slides (or photomicrographs) of retinal tissue from three rats in the acute and subchronic neurotoxicity studies on malathion; and 3) should EPA require the registrant to perform and submit additional neurotoxicity studies to evaluate possible effects of malathion on learning and/or behavior and/or other neurological parameters as exemplified in a literature article by Desi et al. (1976) in which maze performance (learning) and EEG and EMG recordings were reported as being affected in rats treated with malathion. The conclusions of the ad hoc neurotoxicity group are included as Attachment 3 to this document.

The external peer review panel was composed of three toxicologists, Drs. Walter Decker, Michael Dourson and Rolf Hartung. According to the December 22, 1998 HIARC report, each panel member received all pertinent materials (DERs, 1-liner data base and Dr. Dementi's questions and memoranda). They were asked to address multiple questions concerning seven issues. The questions were prepared by Dr. Dementi. There were also three general questions prepared by Dr. Henry Spencer, HED's external peer review coordinator; these questions were not addressed in the December 22, 1998 HIARC report. According to Dr. Dementi (conversation on March 23, 2000), the general questions were included with Dr. Hartung's May 29, 1998 responses. (Dr. Dementi supplied copies of the original panel's responses on March 16, 2000.)

HIARC meetings on August 18, August 20 and August 27, 1998, were devoted to evaluating the external peer review panel's responses/comments and determining how this information would alter the decisions of the November 6, 1997 HIARC meeting. Prior to the 1998 meeting, individual members of the HIARC with expertise in areas of the seven topics (as presented to the peer review panel) were assigned to review the panel responses and present their findings to the Committee. According to the December 22, 1998 HIARC report, "*Dr. Dementi presented an overview of the Panel comments and guided the Committee through each topic. The Committee evaluated the Panel' (sic) responses and the assessments by the individual HIARC member assigned for each topic in conjunction with the malathion toxicology database*."

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possible "conflict of interest" for this malathion assignment (March 1, 2000 meeting).

General Comments on 1997 and 1998 HIARC Reports

1. The 1997 HIARC meeting was held on November 6, 1997. Subsequent to that meeting, an internal (HED) ad hoc neurotoxicity subgroup was formed to address three issues of concern to Dr. Dementi. The subgroup met on November 13, 1997. The December 17, 1997 HIARC report includes the conclusions of the subgroup as an attachment but does not indicate if the HIARC reviewed and accepted the conclusions of the subgroup.

2. The August 1998 HIARC meetings discussed and evaluated the external peer review panel's responses/comments for each question under the seven issues, however, in some instances, the December 22, 1998 HIARC report does not state directly the Committee's conclusions about the panel's findings and the basis for the conclusions.

3. Three general questions were submitted to the external peer review panel, along with multiple questions under each of the seven issues. There is no indication in the December 22, 1998 HIARC report that the general issues were discussed at the August 1998 meetings.

General Comments on Whether HIARC Reports Reflect Dr. Dementi's Scientific Opinions

1. The December 17, 1997 and December 22, 1998 HIARC reports reflect that Dr. Dementi's scientific opinions were addressed and considered as evidenced in the following:

a. After the November 6, 1997 HIARC meeting, an internal (HED) peer review group was formed to address three neurotoxicity issues on which Dr. Dementi differed with the HIARC conclusions. He attended the meeting and presented his positions.

b. His opinions were identified in both the December 17, 1997 and December 22, 1998 HIARC reports as Minority Reports. His detailed letters were attached to the reports.

c. As stated in his November 20, 1997 letter (Attachment 4), he thought the internal ad hoc neurotoxicity subgroup was biased in their opinions on the three issues. Subsequently, these issues, along with five others identified by Dr. Dementi, were submitted to an external peer review panel. He prepared the questions, which were not reviewed or edited by HED management prior to submission to the panel.[4] He also prepared follow-up questions to the panel.

d. Prior to the scheduled August 18, 1998 HIARC meetings to discuss the external peer review panel's responses, Dr. Dementi expressed concern that so many issues were contemplated for one meeting and that each issue would not be accorded the time needed. The record shows that the HIARC met on three days, August 18, 20 and 27, 1998, evidence that more time was allotted in response to his concern.

[4] Confirmed by William Burnam, Chief, Science Analysis Branch, HED, in a March 16, 2000 email message.

2. In his November 5, 1998 letter (Attachment 18), Dr. Dementi states that "...*the external reviewers' conclusions are in many cases complex and are not adequately addressed in brief statements offered as the "Panel's Response" under various questions in the HIARC draft document of October 27, 1998."* He recommended that his "Consolidation of External Peer Reviewer's Comments on Malathion non-Cancer Issues" (Attachment 12) as the preferred assessment of the reviewer's comments. It is this evaluator's opinion that the individual responses from the external peer review panel varied in their usefulness in sorting out the seven issues. The only issue on which there was uniform agreement was that there should be follow-up on the retinal rosette findings (Issue 5). In many instances, the panel did not answer a question directly but instead referred to general toxicological principles. In some instances, their responses implied an misunderstanding of the question and/or EPA's risk assessment process. However, in their defense, some of the questions were complex and possibly inappropriate for an external peer review panel. Dr. Dementi has listed what he thinks are the conclusions supported by *at least* (his emphasis) a consensus of the external reviewers at the end of Attachment 18 of his February 28, 2000 letter. This evaluator does not agree that these conclusions can be supported for the following reasons: 1) the panel offered individual opinions and did not express a collective or consensus opinion; 2) there was total agreement on very few of the responses; 3) many of the questions were not answered directly; and 4) Dr. Dementi misinterpreted some of the panel's responses.

ISSUE 1: Food Quality Protection Act (FQPA) 10X Safety Factor for Protection of Infants and Children[5]

Dr. Dementi's Position as Summarized in February 28, 2000 Letter

The HIARC's decision to delete the FQPA imposed 10-fold safety factor for the protection of infants and children is unsupported by the data base. Reduction or removal of the Congressionally imposed 10X safety factor is conditioned upon: 1) a reliable data base, 2) a complete data base and 3) evidence that young/developing individuals are no more susceptible than adults to the toxicologic effects of the agent in question. ***All*** *of these conditions must be met. Yet, in my witness,* ***none*** *of the conditions are met for malathion. The rationale for removal of the factor as presented in the December 17, 1997 HIARC report (**Att 2**) is inadequate, and there is little evidence the subsequent August 6, 1998 report of the FQPA Safety Factor Committee (t-drive available), also recommending the safety factor's removal, contributed any more definitive evidence. When questioned as to the adequacy of the data base to remove the safety factor, the External Peer Review did not support the factor's removal, based upon the inadequate reliability of the data base to address the susceptibility issue; and the incompleteness of the data base, as evidenced by the need for cholinesterase data in exposed young versus adult animals and additional behavioral effects testing. The External Peer Review Panel characterized a variety of deficiencies and needed studies as data gaps. (**Att 16**) Now whether these deficiencies are data gaps in the strict sense of being unsatisfied end points in Guideline studies (as I believe some in fact are), or inadequacies in the overall assessment of malathion to address health effects concerns, is probably one more of semantics than substance with respect to the intent of Congress to protect infants and children. If there is serious doubt as to the intent of Congress, then ask the Congressional author(s) of the FQPA.*

To the extent the data base is not complete, it is not reliable and vice versa. Additional Guideline data gaps do exist (subchronic inhalation, subchronic cholinesterase in the dog), though these tests do not directly address the question of susceptibility, they do establish the absence of a complete data base in terms of Guideline requirements. In my opinion, as supported by that of the External Peer Review, the data illustrate a need for further behavioral effects testing, a requirement that might be satisfied by the Guideline Developmental Neurotoxicity Study recently being required by OPP for all organophosphates. In the case of malathion, to the extent that a need for such testing has been identified based upon published works which indicate behavioral effects and/or effects on learning and memory at low doses, the requirement for additional behavioral effects testing is therefore more than generic, and thus in effect constitutes another data gap that should be satisfied prior to removal of the 10X factor. The Developmental Neurotoxicity does pertain to the susceptibility issue.

As to the question of differential susceptibility revealed in the malathion Guideline reproduction

[5] In this evaluation, the terms NOEL and LOEL (as opposed to the present terminology of NOAEL/LOAEL) are used as they were in the HIARC documents.

study, I do not accept HIARC's rationale for discounting the actual evidence of enhanced susceptibility of the offspring. I believe my views are well presented in the background materials cited. Furthermore, I have recommended an external re-review of the reproduction study, focused on the differential susceptibility aspect.

*In addition to evidence of increased susceptibility of offspring in the Guideline reproduction study, evidence has been cited of increased susceptibility of the young exposed to malathion [**Atts 17** and **18** (p. 154)] which was not identified in the December 17, 1997 HIARC report. This additional information was also not acknowledged by the committee in its final December 22, 1998 report.*

Parenthetically, though not previously mentioned in the HIARC or FQPA Safety Factor Committee's consideration of malathion, the National Research Council's (1993): "Pesticides in the Diets of Infants and Children" (the report which spawned FQPA) indicates that: "There is speculation that neonates and infants may be more susceptible to chemically induced neurotoxicity, in part because of the immaturity of their blood-brain barrier. Watanabe et al (1990) point out that the central nervous system in developing individuals is potentially vulnerable to chemicals for a protracted period because the central nervous system requires longer than most other organ systems for cellular differentiation, growth, and functional organization. Therefore, any increase in accessibility to cytotoxic agents because of delayed maturation of the blood-brain barrier could have serious consequences." (p. 89) Currently, OPP gathers no data on the relative accessability of cholinesterase inhibitors to the CNS of adult versus young animals. Since cholinesterase inhibition is a most fundamental end point for an agent designed to inhibit that enzyme, differential inhibition in adult versus developing individuals may be expected to be a most sensitive indicator of differential susceptibility. As said previously, and as supported by the External Peer Review, the data base lacks reliability to address the susceptibility issue absent cholinesterase data, particularly in developmental toxicity and reproduction studies.

Cited in Dr. Dementi's February 28, 2000 Letter: 057701ha.002: pp. 6-8; Att 1; Att 2: pp. 48, 50, 57- 64; Att 6: pp. 109-110; Att 8; Att 11; Att 12: pp. 124-126; Att 13; Att 14; Att 15; Att 16; Att 17; Att 18: pp. 148-155.

Additional Information from Dr. Dementi's Detailed Memoranda/Letters

1) From Attachment 6: Letter from B. Dementi dated December 17, 1997

This letter comments on the December 4, 1997 draft report of the November 6, 1997 HIARC report. Under Acute Dietary Risk Assessment, Dr. Dementi comments on the HIARC decision that the 10x FQPA safety factor should be removed. He discusses the findings of the rat and rabbit developmental toxicity studies and concurs with the HIARC that neither of the studies demonstrated evidence of increased sensitivity of developing organisms, insofar as the parameters evaluated were concerned. He then says that there is a serious question about whether such

parameters were adequate to detect critical endpoints. The lowest dose in both the studies was above those that inhibit cholinesterase (ChE) in adult rats and rabbits (assumed to mean in other studies). In the absence of ChE measurements or clinical signs in the developing organisms versus those of maternal animals, it is not possible to affirm that developing organisms were not more adversely affected. It is his opinion that ChE could have been more remarkably inhibited in selected developing tissues of fetuses. Furthermore, a given level of inhibition may be more deleterious in developing organisms.

Concerning the reproduction study, the draft HIARC report states that pups were no more sensitive than adults on the basis of body weight, mortality and clinical signs. The doses in this study were 0, 550, 1700 and 7500 ppm; the low dose is equivalent to 43 mg/kg/day and 51 mg/kg/day in males and females, respectively. Dr. Dementi states that it is his observation that doses of 43-51 mg/kg/day and above have resulted in cholinesterase inhibition (ChEI) in other studies. He says it is not surprising that clinical signs were not observed at the high dose. Rats tolerate ChEI from malathion exposure remarkably well. He reiterates his opinion that developmental and reproduction studies are not of the character needed to differentiate relative sensitivity of young and mature animals to satisfy FQPA concerns. The absence of ChE data is the most fundamental road block for use of these studies. He cites a study by Pope and Chakraborti[6], which he says is evidence that young and developing animals have an enhanced sensitivity to ChE inhibitors in general, attributable to ChEI.

Dr. Dementi notes that in the HIARC document, under Determination of Sensitivity, it states that ChE data were not obtained for maternal animals nor their offspring or fetuses in the reproduction and developmental toxicity studies, without any discussion of the implications of this lack of data.

Under Chronic Dietary Risk Assessment, in response to the section that provides the reasons why the 10x FQPA safety factor was removed, he provides similar arguments as for the acute risk assessment.

2) From Attachment 8: Letter from B. Dementi dated February 10, 1998

This letter comments on the HIARC report for the November 6, 1997 meeting. Under the Reproductive Toxicity section in the report, Dr. Dementi quotes the following, *"Although the offspring NOEL (131 mg/kg/day in males and 153 mg/kg/day in females) was lower than the parental systemic NOEL (394 mg/kg/day in males and 451 mg/kg/day in females), the Committee determined that this was not a true indication of increased sensitivity of offspring because: (I) pup body weight decrements were primarily observed at postnatal day 21; (ii) during that period (i.e., later portion of lactation), young rats consume approximately twice the diet per unit body weight as an adult rat consumes (i.e. 1 ppm in the diet of a young rat is approximately 0.1*

[6] Pope, C. N. and Chakraborti, T. K. (1992) Dose-Related inhibition of brain and plasma cholinesterase in neonatal and adult rats following sublethal organophosphate exposures. Toxicol. 73, 35-43

mg/kg/day whereas in older rats, this ppm level is equal to 0.05 mg/kg/day) and (iii) the estimation of the test substance intake in pre-weaning animals is likely to be more than double the adult intake because of the availability of the test material both via the milk (lactation) and food, particularly after the mid point of lactation." Dr. Dementi states that he has concerns about the reliability one can place in these arguments, lacking definitive data, to conclude that offspring were no more sensitive than adults.

He acknowledges that weight decrements were primarily observed at postnatal day 21, however pup weights decreases were statistically significant on days 7, 14 and 21 for the F_2B generation at the "penultimate" dose (assumed to be the highest dose in the study, 7500 ppm), which the study report concluded to be treatment-related. He states that no record is made of pup food consumption, so it is presumptive to draw conclusions about what pups consumed in the control and treated groups. Generalities regarding relative food consumption of pups versus adults cannot be reasonably used to reach definitive conclusions about chemical exposure in the diet. Furthermore, there are no data in the study to demonstrate the presence or absence of malathion in the milk. He concludes that the reasoning used to dismiss the finding of greater sensitivity of offspring is speculative and not of the definitive character required to refute the positive evidence that pups are more sensitive than adults.

Dr. Dementi then comments on the statement in the HIARC report, " *A two generation reproduction toxicity study in rats showed no increased sensitivity in pups compared to adults.*" He states that FQPA requires the use of an extra 10-fold safety factor unless, on the basis of reliable (his emphasis) data, a different level is determined to be safe for children. He then provides arguments about the absence of ChE data in the developmental and reproduction studies as discussed under Attachment 8. He concludes that the reasoning used by the Committee to dismiss evidence of enhanced sensitivity in offspring in the reproduction study is a violation of the intent of Congress that the 10x factor be discounted only on the basis of reliable data.

3) From Attachment 11: Letter from B. Dementi dated March 20, 1998

This letter concerns the chronic dog study with malathion. This evaluator cannot find any information pertinent to Issue 1, except reference to the FQPA requirement that reliable data be obtained.

4) From Attachment 13: Letter from B. Dementi dated July 29, 1998

This letter concerns comments which Dr. Dourson provided to additional questions posed by Dr. Dementi. The first comment is said to respond to Dr. Dementi's first question. (The July 7, 1998 letter from B. Dementi to Dr. Henry Spencer was supplied by Dr. Dementi on March 23, 2000.) In this question, Dr. Dementi stated that decreased pup weights were seen at days 7, 14 and 21 in the 7500 ppm group of the F_2B generation. The effect at 21 days could be explained by greater food consumption and chemical in the milk. The question is whether this argument can be used to dismiss the findings at days 7 and 14. Dr. Dementi states that Dr. Dourson expresses concern

regarding the reliability of reported pup weights during days 7 and 14 of lactation, which he says are due to chance, but concludes that LOEL/NOEL are 5000/1700 based on body weight changes on day 21 of lactation. Dr. Dementi says it should be noted that the decreases on days 7, 14 and 21 were all statistically significant findings. He states that Dr. Dourson didn't answer his real question, i.e., whether the greater sensitivity of pups in this study can be discounted by the arguments in the HIARC report without demonstrating malathion in the milk and without data on food consumption in pups during lactation.

5) From Attachment 14: Letter from B. Dementi dated August 3, 1998

This letter was written in preparation for the August 18, 1998 HIARC meeting on malathion. It provides additional comments on the two-generation reproduction study (MRID 41583401, DER #5). Dr. Dementi refers to the December 17, 1997 HIARC report, which says, *"For parental systemic toxicity, the NOEL was 5000 ppm (394/451 mg/kg/day in M/F) and the LOEL was 7500 ppm (612/703 mg/kg/day in M/F) based on decreased P generation body weights during gestation and lactation and decreased F1 pre-mating body weight."* He says he has concerns about this finding for the following reasons: 1) parental (dam) body weight was not affected at any dose level during either of the two F_1 lactation period, i.e., for litters F_2A and F_2B; 2) dam body weights were significantly less in the 7500 ppm dose group for both F_0 lactation periods, i.e., for litters F_1A and F_1B. In the case of both F_0 lactation periods, the effects were most remarkable on lactation day 0, an effect which should be properly viewed as a manifestation of effects incurred during gestation and delivery. The meaningful period of assessing dam body weight effects of/during lactation rests on what happens after Day 0, i.e., on days 7, 14 and 21, in this case. He says that his examination of the data in the F_0 lactation periods reveals that there is recovery of body weights by day 7. Body weight changes assessed across the 21-day period (e.g. days 7-14, 7-21 and 14-21) in all dose groups appear essentially unaffected at any dose level. He notes that this if without benefit of statistical analyses, which he recommends should be done. He says that the reason why the body weights in the 7500 ppm group are less than control post day 0 is due to a carry over of the Day 0 deficit, since there is little or no evidence of body weight decreases at any other time point. It is his view that dams in the 7500 ppm dose group were affected during pregnancy, as indicated by the weight decreases on lactation day 0; there is no evidence to show that weights were affected during lactation; 3) decreases in dam body weight during gestation cannot be interpreted as uniquely parental/dam effects; 4) during the pre-mating period, there were no effects on F_0 male or female body weights, however there were decreases in both F_1 males and females at 7500 ppm during the pre-mating period. The F_1 animals, unlike the F_0 animals, were exposed to malathion in utero and hence effects cannot be separated from a possible fetal/developmental etiology. He concludes, *"...the fact that body weight effects were observed in the F1 animals at 7500 ppm during premating, but not in F0 males or females during premating is supportive of a possible adverse effect of the test material on F1 animals during development, manifested as an enhanced adult sensitivity."*

He states that closer examination of the DER does not reveal any indisputable or *reliable* (his emphasis) evidence that body weight changes in adults at any dose level, either during gestation,

lactation or pre-mating periods, as claimed in the DER report. He invites EPA experts in reproduction toxicology to examine the study closely and comment on his views in this letter.

6) From Attachment 15: Letter from B. Dementi dated August 10, 1998

This letter is a follow-up to the August 3, 1998 letter concerning the body weight changes in parental animals. Dr. Dementi says that there was no adverse effect of malathion on body weight changes during the gestation, lactation and premating periods, as claimed in the December 17, 1997 HIARC report. This means that the parental NOEL is $\geq$7500 ppm, while the developmental NOEL/LOEL is 1700/5000 ppm. He states that the study author concluded there was no adverse effect on parental animals. He thinks the principal reason for the discrepancy between the DER and the study report is that the DER reported only body weight effects, whereas the study report considered body weight changes. While the body weight in the 7500 ppm parental group was less than the control, body weight changes were unaltered. He notes that the study report says that mean weekly weight data for males and females during the pre-mating period were lower than control and these differences were statistically significant. Mean weight gains over the entire 10-week premating period for both sexes were comparable to control data. Dr. Dementi thinks there is a need for revisions to the DER (#5) to present a more satisfactory interpretation of the findings.

He compares the findings for the F_0 and F_1 males. Mean body weights during the mating and post-mating periods for F_0 males were comparable to the controls. By contrast, the F_1 males (exposed in utero to malathion) had statistically significant decreased body weight in the 7500 ppm group during the mating and post-mating periods that are consistent with the lower weights seen in this group during the premating period. He quotes from the study report, *"Thus, no adverse effect of treatment up to a dietary level of 7500 ppm was indicated from weight gain data for males during the mating and post-mating intervals for either the P1 or F1 generations."*

He concludes that this means there is a larger gap between the developmental NOEL/LOEL and the parental NOEL to be explained by the HIARC in removing the 10x FQPA safety factor.

7) From Attachment 16: Letter from B. Dementi dated August 17, 1998

This letter concerns the format of the August 18, 1998 HIARC meeting. Dr. Dementi states that he is concerned that the discussion will be restricted to the eight topics submitted to the external peer review panel. He says there were preliminary questions responded to by the three scientists on the panel. The questions pertained to the acceptability of the various malathion DERs, whether critical effects were chosen in the various studies and whether the data base is complete. He indicates that one of his principal concerns, as expressed in December 17, 1997 letter, is whether there are gaps in the malathion data base.

Dr. Dementi then extracts quotes from the three external peer review panel members, which he says pertain to the acceptability of the malathion data base. The quotes attributable to the

panelists are extracted from this letter and presented in Attachment 4. This evaluator notes that the quotes are taken out of context from various questions concerning the eight issues and are not direct responses to the question about the completeness of the malathion data base.

Dr. Dementi states that the views of these external scientists serve to underscore his opinion that the toxicology data base is not complete, as claimed in the December 17, 1997 HIARC report. He says that he doubts that Congress intended anything other than fully acceptable studies, with no data gaps of the nature identified by the external reviewers, be used to satisfy the criterion of a complete data base for removing the 10x safety factor.

Concerning the format of the upcoming meeting, he expresses concern that one meeting will not be sufficient time for him to express his views on the malathion issues. (The HIARC met on August 18, 20 and 27, 1998.) He also objects to the assignment of certain members of the HIARC to certain questions, as this may have a negative effect on the extent to which other members of the Committee evaluate all the issues, i.e., too much reliance of the Committee as a whole may be placed on the opinions of one principal reviewer. He also requests that he be given the opportunity to provide follow-up after the meeting so that he has time to reflect on issues that may come up during the meeting.

8) From Attachment 17: Letter from B. Dementi dated September 24, 1998

This letter forwards a copy of a journal article by Mendoza[7] offered for inclusion under the "Information from the open literature" section of the HIARC report as relevant to the determination of sensitivity for FQPA considerations. Dr. Dementi does not provide any of the details of the article. He states that he has read the article and it leads him to conclude that it provides information indicating that younger animals are more sensitive to malathion, but he has not had time to review it.

9) From Attachment 18: Letter from B. Dementi dated November 5, 1998

This letter comments on the October 27, 1998 draft report of the August HIARC meetings. A copy of the draft report was supplied by Dr. Dementi on March 23, 2000.

Under P.5, paragraphs 5 and 6, Dr. Dementi states that the report does not provide the panel's response or the HIARC's conclusion relevant to question 3)c), i.e., is the data available in the developmental study sufficiently reliable (his emphasis) to discount the 10x safety factor as required under FQPA. He says the panel's opinion was unanimous that it was not.

Under P.6, paragraph 2, last line, concerning the statement, *".....evidence (of parental toxicity) is not strong"*, he states that if the evidence is not strong, how can it satisfy as reliable data for the

[7] Mendoza, C.E. (1976) Toxicity and Effects of Malathion on Esterases of Suckling Albino Rats. Toxiol. Appl. Pharmacol., 35, 229-238.

protection of infants and children under FQPA. In his view, which he says was expressed at the HIARC meeting, the study does not show a parental effect at any dose level. This means that the pup weight effects at two doses in absence of parental toxicity establishes a greater sensitivity in the young and developing individuals. He states that it is his understanding from the meeting that Dr. Steve Dapson would complete a review of this study since it was pointed out that the study author had also concluded there were no parental effects at any dose level. This conclusion was based on body weight gain data, which had not been incorporated into the DER. In addition, the study author concluded that offspring were adversely affected at the top two doses.

Under P.6, paragraph 5, he comments on a statement in bold, which he says is not consistent with the tenor of the discussion at the August 20, 1998 meeting. He asks if there has been other meetings of the HIARC since August 27. The paragraph says, *"The presence of the chemical in the milk is a generic assumption ....."* He states that the report must show that, at the August 18 meeting, Dr. Alberto Protzel left to retrieve a residue chemistry metabolism study in the goat. According to Dr. Dementi, that study showed that only two non-cholinesterase inhibiting metabolites of malathion, i.e., malathion was not present in the milk. He says he subsequently spoke to Mr. Bill Smith, the malathion team chemist, who confirmed that malathion is not a residue in milk. Dr. Dementi concludes that the generic assumption about chemical in the milk does not apply in this case. He asserts that this information should be in the HIARC report. In addition, he maintains that the HIARC needs to revise its conclusion in the use of the milk argument to discount increased sensitivity of young in the reproduction study.

Under P.6, last paragraph, concerns referring a decision about the FQPA safety factor back to the FQPA Safety Factor Committee. Dr. Dementi doesn't affirm the reasoning that the HIARC is not responsible for determining the FQPA 10x Safety Factor. If true, the chemical should be referred back to the FQPA Safety Factor Committee.

Under P.7, paragraph 3, he comments on the reproduction study and asks if it meets the test for *reliable* (his emphasis) data for the protection of infants and children under FQPA. He maintains that the expert panel said no. The HIARC report acknowledges limitations of the study protocol to assess increased susceptibility.

Under P.7, between paragraphs 4 and 5 concerning the missing Panel's Response to question 3. He says the panel's response should be recorded as Dr. Dourson suggesting a 3x safety factor, while acknowledging 10x may be useful as a management tool. Drs. Hartung and Decker said no, though Dr. Hartung insisted offspring must be shown to be less sensitive. Also, he states the panel members were not aware of the study author's conclusion about body weight gain data not shown in the DER. Nor were they aware that malathion has been shown to not be present in milk.

Under P.7, paragraph 5, Dr. Dementi says the report needs to be clear that only developmental and reproduction studies assess the relative sensitivities of young and adult animals. The external panel said that the reproduction study does not provide reliable data. This, taken in concert with data showing greater sensitivity in young animals, leads him to doubt that the public would take

comfort in the generic issue argument to discount the absence of satisfactory data.

Under P.8, paragraph 5, line 5, Dr. Dementi says that Dr. Dourson's response should appear as "*..... principally because the critical **effect** was not monitored in the two-generation reproduction study in a potentially sensitive subgroup (i.e. young rats).*" He also quotes Dr. Dourson as saying, *"The lack of the monitoring of the critical effect in the developing offspring, and specifically, the lack of such measurement of RBC cholinesterase inhibition in the 2 generation study is a data gap that can best be addressed through the use of a 3-fold uncertainty factor when determining the RfD."* Dr. Dementi says it's important to make the audience aware of the identity of the critical effect (ChEI) because it is the basis of the chronic RfD and that Dr. Dourson considers it a data gap. The remainder of this paragraph concerns the chronic RfD and will be addressed under Issue 3.

P.11, paragraph 2, concerns whether a sentence about the FQPA Safety Factor being neither applicable or appropriate for this study should be bolded. Dr. Dementi states that to do so casts aspersions on the appropriateness of the question of whether the chronic toxicity/carcinogenicity study weighs at all in the decision to retain the FQPA 10x safety factor. He states that he is troubled by statements such as, *"At present the determination of susceptibility is made not based on the results of one study (where in fact one appropriate study that is positive will do) but rather on a weight-of-evidence (emphasis added) basis that includes acute and subchronic neurotoxicity studies, the prenatal developmental toxicity studies in rats and rabbits, the 2-generation reproduction toxicity study in rats as well as the toxicity profile of the chemical (emphasis added)."* He states that he puts this question forward to make it *transparent* (his emphasis) to observers that this major study does not contribute anything magical to the claim of weight-of-evidence toward justifying removal of the 10x safety factor.

Under General Comments, 4), Dr. Dementi states that, at the August meeting, he referred the HIARC to two studies in the one-liner data base that showed young animals are more sensitive than adults to malathion. The studies include: 1) acute oral study in which the LD_{50} for malathion (95% a.i.) was 80 mg/kg in the calf and 560 mg/kg in the cow; 2) an acute intraperitoneal study in male rats in which the LD_{50} for malathion (assumed 99% from another study) was 750 mg/kg in adults and 340 mg/kg in the weanling. He states that there is no acknowledgment of this discussion in the HIARC minutes. He also refers to a 1963 study by Brodeur and DuBoise which concluded that young animals appear to be more susceptible to malathion than older animals. He mentions the Mendoza study referred to in Attachment 17. He doesn't know if the study has been formally reviewed. The study concluded that 1 day-old Wistar rats were found to be nine times more susceptible to malathion than 17 day-old pups. The LD50 was 209 (177-250) mg/kg for the 1 day-old rats as compared to 1806 (1415-2003) for the 17 day-old rats.

Under General Comments, 5), Dr. Dementi states that the completeness of the malathion data base was addressed by the external peer review panel but was not discussed at the August HIARC meetings. He says that the external reviewers identified several data gaps or data deficiencies summarized in this letter. He then states, " *Now whether these deficiencies are data gaps in the*

strict sense of being unsatisfied end points in Guideline studies (as I believe some are), or inadequacies in the overall assessment of malathion to address health effects concerns, is probably one more of semantics than substance with respect to the intent of Congress to protect infants and children. A most notable statement along these lines was made by Dr. Dourson, who wrote: "I am not satisfied that the potential risk to humans is addressed with the data available in this review package." (P. 3 of his June 3, 1998 comments). ***So the point I am making here is that it cannot be claimed by HIARC that the no-data-gap qualifier required under FQPA for removal of the 10X safety factor has been met.***

Under General Comments, 6), Dr. Dementi states that the HIARC has used the same reasoning employed at the November 1997 meeting to refute the conclusions/recommendations of the expert panel. He says it is not clear why the issues were referred back to the HIARC, but all of the Committee's decisions require review and confirmation outside HED before they become "regulatory acceptable". (Confusing to this evaluator as to what organizational unit outside HED would be reviewing and confirming the HIARC decisions.) **He states that the following conclusions are supported by *at least* (his emphasis) a consensus of the external reviewers who had the full data package in hand.**

a) An acute (one-day) endpoint as high as 0.50 mg/kg is not supported by the data base. (Assumed to be 50 mg/kg, which was the dose for the endpoint.)

b) It cannot be interpreted that the developmental and reproduction studies provide *reliable* (his emphasis) information to discount the 10x FQPA safety factor because the studies did not compare either ChE or behavioral measures in adult and young/developing animals.

c) The finding of increased sensitivity of pups vs. adults in the reproduction study confirms the retention of the 10x FQPA safety factor.

d) There is no NOEL for ChEI for females in the combined chronic toxicity/carcinogenicity study, given that there was evidence of a post 3 month recovery of RBC ChE in females. Therefore, in the absence of an additional uncertainty factor, this dose cannot serve as the basis for the RfD.

e) ChE methodology may have been a problem in this study. (Assumed to refer to combined chronic toxicity/carcinogenicity study.)

f) Use of the rat study rather than the human study is unsupported.

g) Use of a "mere" 10x safety factor to allow for "uncertainties" (knowing the lack of carboxylesterase in human plasma) for interspecies variability is inadequate if the rat study is used.

h) The UF to be applied to the inhalation endpoints (intermediate and long term) to compensate for the absence of a NOEL for nasal and laryngeal degeneration/hyperplasia is 10x.

I) A consensus exists among the external reviewers that additional assessment of some sort is indicated to address the absence of NOELs in the inhalation study.

j) Retinal tissue histopathology slides from those animals identified in the DER, along with slides from the lower dose groups, should be submitted for independent pathology assessments.

k) Additional behavioral effects testing, e.g., developmental neurotoxicity study, should be required.

l) Additional testing in animal models should be required to quantitate any gender specific disparity with respect to ChEI.

Evaluation by External Peer Review Panel

The external peer review panel was asked three questions concerning Issue 1 (Issue II in their questions). The first question asked if the evidence indicating greater sensitivity of offspring versus parental animals in the two-generation rat reproduction study can be dismissed as "...not a true indication of increased sensitivity of offspring..." for the reasons stated in the 1997 HIARC report. Dr. Decker said simply, *" No, because some toxic effects have been reported."* Dr. Dourson responded that the answer depends on how the doses were calculated in the HIARC report. If the doses for the offspring were simply a reflection of the adult doses (which they appear to be), then the apparent greater sensitivity of offspring is offset by their likely higher ingested dose. Thus, the apparent sensitivity can be discounted based on the reasoning in the HIARC report. However, if the offspring dose is calculated from their own food consumption, then the apparent toxicity is real and should not be discounted. Additional comments from Dr. Dourson in response to a follow-up question from Dr. Dementi are presented in the above discussion of Attachment 13. Dr. Hartung said, "*I concur with the reasoning of the Committee. But I do not concur with the ultimate conclusions with respect to safety (uncertainty) factors to be used. Whether neonates are more sensitive to a given dose of pesticide (mg/kg basis) is an important, but not the only issue. The newborn are known to have higher food intakes (on a body weight basis) than adults. The higher intake is the consequence of higher metabolic rates, due to increased heat loss determined by surface area/body weight of the neonate, and due to increased food intake due to growth requirements. For foods with equal pesticide residues the child will acquire a higher dose of pesticides than the adult (see NRC, 1993; Pesticides in the Diets of Infants and Children). Given this circumstance, it is necessary to demonstrate that the neonate is less sensitive (not equal to) than the adult on a mg/kg basis, or that food pesticide residues are being managed in such a way as to restrict the quantities of residues likely to be found in the diet of children as compared to adults."*

In his consolidation report (Attachment 12 of February 28, 2000 letter), Dr. Dementi summarizes the peer review panel's responses as follows:

"*Dr. Dourson: Yes, to the extent that the dose in offspring is not derived from actual assessment*

of food intake.

Dr. Hartung: Yes, but expresses the view that neonates must be shown to be less sensitive than adults (not equal to) before the FQPA 10X safety factor can be deleted.

Dr. Decker: No, "because some toxic effects have been reported."

In his comments section, Dr. Dementi says, *"Two reviewers say yes (with qualifying remarks) and one says no. I had hoped the reviewers would say something specific about views expressed in Ref. F, supported by data in Ref. G (selected pages from the study report). The point is that an effect on pup body weight occurred at a dose below that which similarly affected dam body weight. The effect on pups was dismissed by the Hazard ID Committee as evidence of greater sensitivity of pups for reasons which in my view were unsubstantiated, i.e. no proof of the presence of malathion in the milk, nor any evidence of how much food pups may have consumed under circumstances wherein malathion in the diet may have influenced food intake. It may not have been clear to the external reviewers that the presence (let alone the amount) of malathion in the milk has not been shown by analysis. It should also be noted that while pup body weight changes were seen during lactation days 7 (where pups rely essentially exclusively on milk), 14 and 21 in the 5000 ppm dose group (the NOEL for dam body weight change in the study at large), dam body weight changes were not apparent during the lactation period even at the top dose of 7500 ppm. Hence, during lactation pup NOEL/LOEL = 1700/5000 ppm, while dam NOEL $\geq$ 7500 ppm (HDT). Pope and Chakraborti (1992) (Ref. E) say that young mammals are remarkably more sensitive than adults to numerous organophosphates. Hence, the burden is not light to justify dismissing evidence of a more selective effect in pups due to exposure to this particular OP."*

The December 22, 1998 HIARC report summarized the panel's responses as, *"Two panel members stated that there is evidence indicating greater sensitivity (with qualifying remarks) while one stated that there is no indication for greater sensitivity."*

In summary (evaluator's opinion), the question was whether evidence of greater sensitivity, which was presented as a given fact, can be dismissed based on the HIARC's reasoning. Two panelists said yes to this question, not that there is evidence of greater sensitivity, which was acknowledged in the question. Although Dr. Decker answered the question directly by responding no, more elaboration on what toxic effects he refers to would have been helpful.

The second question asked if the data from the reproduction study can be considered adequate to address whether young or mature animals are more sensitive to malathion, given the absence of ChE and behavioral assessments in adult and young animals. Dr. Decker said no because more behavioral (and learning) testing should be performed. He also noted that the FIFRA guidelines should be updated since more information (presumably on behavioral/learning testing) has emerged in the last decade. Dr. Dourson said no, the data are absent. He said perhaps the argument could be made, based on an analogy to other ChE inhibitors, that young animals in a

reproduction study are not as sensitive, or equally as sensitive, as adults from a subchronic study or a 2-year bioassay, but the argument is not made in the materials provided for his review. Dr. Hartung said, *"The comparison of relative sensitivities is only meaningful when data exist that were collected under comparable conditions. It is obviously possible to make many comparisons, as long as comparably derived data exist. However, because toxicity testing is inherently open ended, a question of this type can never be answered with certainty in detail, although general comparisons are possible."*

In his consolidation report (Attachment 12 of February 28, 2000 letter), Dr. Dementi summarized the panel's responses as follows:

"Dr. Dourson: No.

Dr. Hartung: Seems to say no since the data in question do not exist. Though at this point he does not actually affirm the critical importance of the data in question, he attests to the importance elsewhere in the document. For example, in defending the use of the human cholinesterase study, Moeller and Rider, he says: "....it addresses a diagnostic end-point that is known to be mechanistically related to the toxicity of OPs." (p. 8); and "Changes in some behavioral parameters that have a degree of correspondence to acetylcholinesterase, in particular to brain cholinesterase, would be expected." (p. 5)

Dr. Decker: No. Says more behavioral (learning) tests should be performed. FIFRA Guidelines need updating."

In his comments, Dr. Dementi concludes that the external reviewers agree in saying that the data in the 2-generation reproduction study are not adequate to address the question of relative sensitivity of young versus mature animals.

The December 22, 1998 HIARC report summarized the panel's responses as, *"The panel appears to agree in saying no to this question, i.e., data in the 2-generation reproduction study are not adequate to address the question of relative sensitivity of younger versus mature animals."*

In summary (evaluator's opinion), Drs. Decker and Dourson responded no. Dr. Hartung did not answer the question concerning the malathion reproduction study but responded generally. Therefore, it cannot be concluded that he answered no to this question.

The third question asked if the reproduction study provides the reliable (Dr. Dementi's emphasis) evidence of no increased sensitivity in pups as compared to adults required under FQPA to discount the 10x safety factor. Dr. Decker said no, because the evidence is quite thin. Dr. Dourson said that the failure to measure the critical effect in a potentially sensitive subpopulation necessitates the use of an additional uncertainty factor (UF) for data base deficiencies. He recommended that the value of this factor should be 3-fold, in keeping with previous EPA decisions for the magnitude of such data base deficiencies. He repeated his comment under

question 2 about comparing ChEI between young and adult animals for similar chemicals to possibly discount the use of an additional factor. He also repeated his opinion that the FQPA safety factor is not appropriate for the discussion of scientific uncertainties used to establish an RfD. He said, as a risk management tool, the FQPA 10x may be useful for malathion. However, the use of an additional 3-fold factor for data base deficiencies precludes the use of an FQPA factor for scientific reasons. Dr. Hartung said that this is not the correct question. The appropriate question should be whether this study provides clear evidence of less sensitivity among pups compared to adults *"for reasons cited elsewhere in this report".* (The section of the report where this is cited is not identified.)

In his consolidation report (Attachment 12 of February 28, 2000 letter), Dr. Dementi summarizes the panel's responses as follows:

"Dr. Dourson: Suggests 3X as opposed to 10X safety factor. Although, he acknowledges 10X may still be useful as a management tool.

Dr. Hartung: No. Expresses view that the study shows no clear evidence of <u>less</u> sensitivity of offspring, which he considers essential.

Dr. Decker: No. "....evidence seems quite thin." (p. 5)"

In his comments, Dr. Dementi says that the weight of opinion is that the 10X safety factor under FQPA cannot be dismissed.

The December 22, 1998 HIARC report summarized the panel's responses as, *"One panel member suggested a 3x safety factor as opposed to 10x, while acknowledging that the 10x may still be useful as a management tool. The other two panel members said no, though, one member argued that the offspring must be shown to be less sensitive.*"

<u>In summary</u> (evaluator's opinion), Drs. Dementi and Dourson answered no. Dr. Hartung said the appropriate question should be <u>whether</u> this study provides clear evidence of less sensitivity among pups. He doesn't say that the study shows no clear evidence of less sensitivity of offspring. In fact, he doesn't answer the question directly and cites other comments in his report but does not identify where they can be found.

A question relevant to the FQPA 10x Safety Factor was included under question 3 of Issue 2. The panel was asked if the data available in the developmental toxicity studies are sufficiently reliable to discount the 10x safety factor. Dr. Decker said no, since younger animals (and presumably younger humans) are usually more sensitive to toxic effects of any chemical, the 10x safety factor should not be discounted. Dr. Dourson did not respond directly to this question. Dr. Hartung said the available information does not support the deletion of the FQPA 10x Safety Factor. See Issue 2, question 3 for Dr. Dementi's consolidation of the panel's responses to this question.

December 22, 1998 HIARC Report

Concerning question number 1, the HIARC concluded:

In the two-generation reproduction study, for parental systemic toxicity, the LOEL was 7500 ppm (612 mg/kg/day in males and 703 mg/kg/day in females) based on decreased body weights in F_o generation during gestation and lactation and decreased body weight in F_1 during pre-mating. For parental systemic toxicity, the NOEL was 5000 ppm (394 mg/kg/day in males and 451 mg/kg/day in females).

The HIARC concurred with the NOEL/LOEL established by the reviewer in the Data Evaluation Record and reaffirmed the initial conclusion that the adult body weight gain data are confirmation of parental toxicity although it is recognized that the weight-of-evidence is not strong since there is lack of concordance between generations, and because the dose response is not pronounced. Nevertheless, the body weight decrements in F_0 females during gestation and lactation are valid and related; the weight decrements established in gestation are maintained during lactation, and can be attributed to maternal toxicity rather than to factors related to the pregnancy, such as litter size or weight.

The lack of a "significant" body weight gain difference during lactation is not sufficient evidence to discount the statistically significant decreases in mean body weight that were observed. Although the decreased body weight values of F_1 males, without concurrent body weight gain deficits, are not strong evidence of toxicity since F_1 weanling pups were significantly smaller, it was also noted that the males did not regain any of the weight deficits initiated in early life. If there were a total lack of parental toxicity at the highest dose tested, the body weight gains of the males may have demonstrated some recovery. Also, it was noted that the body weight data of F_1 females also indicate significant body weight decrements on weeks 1, 8, and 11, but not week 4 (other weeks were not reported). Therefore, the overall conclusion of the Committee was that parental toxicity was demonstrated by the body weight decrements observed.

It was also noted that the treatment level at which parental body weight decrements were observed was substantially (10-fold) greater than the treatment levels at which cholinesterase inhibition was seen in the chronic rat study with malathion. Although cholinesterase measurements are not recommended by the guidelines, and were therefore not performed, it is assumed that cholinesterase inhibition was indeed occurring in the parental animals which were maintained on test substance for at least 10 weeks premating and through approximately 8 additional weeks of reproductive life. This assumption is made because of cholinesterase inhibition observed in subchronic (13-weeks of dietary administration) and chronic studies with rats.

For offspring toxicity, the NOEL was 1700 ppm (131/153 mg/kg/day in males and females) and the LOEL was 5000 ppm (394 mg/kg/day in males and 451 mg/kg/day in females) based on decreased F_{1a} and F_{2b} pup body weights during lactation.

At the November 7, 1997 meeting it was determined that even though the offspring NOEL (131/153 mg/kg/day in M/F) was lower than the parental systemic toxicity NOEL (394/451 in M/F), this was not a true indication of increased susceptibility since: (I) pup body weight decrements were primarily seen at postnatal day 21; (ii) they are likely related to higher consumption of treated feed in late lactation; (iii) there is an assumption that malathion was present in the milk; and (iv) the pups were exposed to the compound both via the feed (at a high relative intake level) and the milk during late lactation, and were receiving an exaggerated dose of the test substance.

The, HIARC reaffirmed its previous conclusion that there is no increased susceptibility and that even though "quantitatively" there appears to be increased susceptibility based on the NOELs/LOELs. "Qualitatively" the "apparent" susceptibility is due to the assumed higher consumption of treated feed in late lactation and the assumed presence of malathion in the milk. *The presence of the chemical in the milk is a generic assumption made during hazard assessment for all chemicals (unless we have data to show otherwise), and is not unique for malathion.*

Under the current HED Standard Operating Procedures, the HIARC is not responsible for determining the retention, reduction or removal of the 10x safety factor. That determination was made by the FQPA Safety Factor Committee on June 15, 1998. The FQPA Safety Factor Committee evaluated the hazard and exposure (dietary, drinking water and residential) data and concluded that the 10x safety factor for the protection of infants and children (as required by FQPA) should be removed due to 1) completeness of the toxicology database; 2) lack of increased susceptibility in developmental and reproductive toxicity studies; and 3) the use of adequate data (actual, surrogate, and/or modeling outputs) to satisfactorily assess dietary exposure and screening level drinking water as well as residential exposure assessment.

Concerning question number 2, the HIARC concluded:

The adequacy of the two-generation reproduction study to assess increased susceptibility is a generic issue, applicable to all chemicals, and not specific to malathion. At present the determination of susceptibility is made not based on the results of one study but rather on a weight-of-evidence basis that includes acute and subchronic neurotoxicity studies, the prenatal developmental toxicity studies in rats and rabbits, the 2-generation reproduction toxicity study in rats as well as the toxicity profile of the chemical. The HIARC, in previous deliberations, has determined that, based upon the weight of the evidence, a developmental neurotoxicity study (which assesses behavioral effects in the offspring, as well as many other endpoints, and could potentially include cholinesterase inhibition for perinatal animals) would not be required for malathion at this time.

Concerning question number 3, the HIARC concluded:

The HIARC determined that the two-generation reproduction study submitted in support of

malathion reregistration provided adequate and reliable data regarding reproductive toxicity and offspring effects, according to Agency guideline recommendations (83-4) and Good Laboratory Practices. The hazard and dose-response assessments are considered by the FQPA Safety Factor Committee along with the dietary (food and water) as well as residential exposure assessment during risk characterization in order to arrive at a determination of whether or not to recommend retention of the 10x FQPA Safety Factor. ***This determination cannot be made based upon the hazard assessment of a single toxicity study****.*

Evaluator's Comments on Accuracy, Clarity and Transparency of HIARC Reports Concerning Issue 1

1. The HIARC's conclusion in the December 22, 1998 report is expanded from the December 17, 1997 report and provides more details on the body weight/body weight gain effects in parental and young animals. More explanation is provided for the basis of the HIARC's conclusions about these parameters.

2. The following are unclear in the 1998 document:

a. In the first paragraph, it states that the LOEL was 7500 ppm based on decreased body weights in the F_0 generation during gestation and lactation and decreased body weight in the F_1 generation during pre-mating. It is assumed that the effects in the F_0 generation refer to females since they occurred during gestation and lactation, however it is unclear if the F_1 effects were observed in both sexes. The third paragraph discusses decreased body weight values in F_1 males but doesn't address if F_1 females were also affected.

b) The fourth paragraph notes that parental body weight decrements were observed at substantially higher (10-fold) doses than ChEI in the chronic rat study. The report should state the dose from this study. (In the combined chronic toxicity/carcinogenicity study in the rat, the LOEL, based on plasma ChEI, was 29 mg/kg/day.)

c) The fourth paragraph states that it was assumed that ChEI occurred in the parental animals because of ChEI in subchronic and chronic rat studies. Citing the specific studies and doses would make the document clearer.

Evaluator's Comments on HIARC Reports Reflecting Dr. Dementi's Opinions on Issue 1

Dr. Dementi presents three issues (with sub-issues) in his February 28, 2000 letter, as interpreted by this evaluator:

1) evidence of increased susceptibility of young animals, as demonstrated in the reproduction study

a) NOEL/LOEL for body weight/body weight gains in parental animals

b) dismissal of quantitative sensitivity between maternal and offspring effects on the basis of increased malathion exposure to young animals due chemical in the milk and increased food consumption

c) data from the literature attesting to increased sensitivity of young versus animals to malathion

2) complete data base

The external peer review panel was asked if the data base is considered complete (question 3 of general questions to the panel). Dr. Decker said that, except as noted in his reviews of the individual DERs, the data base is complete, although the extent of the literature search on the chemical is not clear. Dr. Dourson said the lack of monitoring of the critical effect in the developing offspring, and specifically, the lack of RBC ChEI data in the reproduction study, is a data gap that can best be addressed through the use of a 3-fold UF when determining the RfD. He states, *"The use of this factor is well within EPA's judgment in developing RfDs, when an otherwise complete data base suggests an otherwise unmonitored endpoint that may end up being the critical effect (the effect on which the RfD is based)."* Dr. Hartung said, *"As for completeness of the data base, in my opinion toxicological studies are inherently open-ended. Regardless of the existing mass of toxicological data, there are always additional questions that can be asked and the data base be further expanded, practically ad infinitum. Thus, there is* ***NO*** *compound that has a complete toxicological data base (I am including substances such as sugar and table salt here)."*

In summary (evaluator's opinion), Dr. Decker said yes. Dr. Dourson said the lack of ChE measurements in the reproduction study is a data gap. Dr. Hartung didn't answer the question directly.

3) reliable data base

This evaluator consulted with Dr. Dementi on March 23, 2000, to discern his definitions of the terms "complete" and "reliable", as describing the malathion data base. He said the terms come from the FQPA legislation. His interpretation is that a complete data base means all the guideline toxicology studies have been submitted. He includes a study which measures learning/behavioral/cognitive effects, such as a developmental neurotoxicity study, with the required studies. His definition of a reliable data base is one in which the toxicology studies measure all the critical endpoints. For malathion, this includes ChE measurements. He stated that the terms were interconnected, in that if a data base is not complete, it is not reliable.

The HIARC and FQPA Safety Factor Committee reports address Dr. Dementi's concerns as follows:

1) Concerning the reproduction study:

a) The December 22, 1998 HIARC report provides more detail in explaining the body weight and body weight gain data than the December 17, 1997 report. Therefore, it can be safely assumed that these data were re-evaluated, as Dr. Dementi understands was supposed to be done after the August meetings. (See discussion of Attachment 18.)

b) The issue of whether there are data to show malathion does not appear in the milk (based on ruminant studies) is not addressed in the December 22, 1998 HIARC report. If the HIARC rejected this argument, it should be addressed in the document. Concerning increased food consumption of offspring, Dr. Dementi's view is that generalities regarding relative food consumption of pups versus adults cannot be reasonably used to reach definitive conclusions about chemical exposure in the diet (Attachment 8). The August 6, 1998 FQPA Safety Committee report states that estimation of the test substance intake in pre-weaning animals is likely to be more than double the adult intake. The basis for this conclusion is not given.[8]

c) In Attachments 17 and 18, Dr. Dementi refers to studies from the open literature which indicate increased sensitivity of young versus adult animals. If these data were discussed at the August 1998 HIARC meetings, they should be included in the report, along with conclusions about the Committee's evaluation of their contribution to the hazard assessment.

2) Concerning the completeness of the data base, as applicable to the FQPA safety factor determination, the December 22, 1998 HIARC report defers to the June 15, 1998 FQPA Safety Factor Committee meeting (report dated August 6, 1998). The August HIARC meetings occurred after the FQPA Safety Factor Committee meeting. Since the question of the completeness of the data base was one of the questions submitted to the panel, it should have been discussed at the HIARC meeting. The Committee's conclusions about the panel's responses and how they impacted the FQPA safety factor determination should have been stated in the December 22, 1998 HIARC report. The issue of how a developmental neurotoxicity study required under the Data Call-In for organophosphates influences the safety factor is an EPA policy and not applicable only to malathion.

3) Concerning the reliability of the data base, which Dr. Dementi interprets as absent critical endpoints in studies, the December 22, 1998 HIARC report explains that the determination of susceptibility is made on a weight-of-the-evidence basis from multiple studies under the conclusions to question 2. Concerning the reproduction study, the HIARC report states, under

[8] Believed by this evaluator to be based on comparison of grams of food consumed per day by young and older rats, 10 versus 20, in publication from the Food and Drug Administration, *Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics*.

conclusions to question 3, that the Committee concluded the study provided adequate and reliable data regarding reproductive toxicity and offspring effects, according to Agency guideline requirements and Good Laboratory Practices.

ISSUE 2: Hazard Identification/Acute Oral (One-Day)

Dr. Dementi's Position as Summarized in February 28, 2000 Letter:

HIARC has set the Acute RfD at 0.05 mg/kg/day despite the fact that in addition to myself, all members of the External Peer Review Panel say it is not supportable, principally due to the absence of cholinesterase activity assessments in the critical study (developmental toxicity study), where body weight change, a relatively insensitive parameter, serves as the basis of the end point. The HIARC decision assumes in the absence of actual data that cholinesterase inhibition, or another more sensitive or serious parameter, e.g. behavioral effects, would not be affected after a single dose of this magnitude. I do not accept that a developmental toxicity study provides sufficiently rigorous data to serve as the basis for defining this critical end point.

Cited in Dr. Dementi's letter*:* **057701ha.002:** pp. 4-5; **Att** 1; Att **2:** pp. 50-52; **Att 6:** pp. 108-109; **Att 12:** p. 124

Additional Information from Dr. Dementi's Detailed Memoranda/Letters

1) From Attachment 6: Letter from B. Dementi dated December 17, 1997

This letter comments on the December 4, 1997 draft report of the November 6, 1997 HIARC meeting. (The draft document was not included with materials provided to this evaluator.) Dr. Dementi states that the LOEL/NOEL of 50/25 mg/kg/day based on decreased maternal body weight gain in the developmental rabbit study was conditional in the DER because individual animal data were not included with the study report. He does not know if these data have been reviewed. He indicates that a non-statistically significant decrease in body weight gain at 25 mg/kg/day could not be adequately evaluated due to the missing data. He concludes that the data should be more closely examined before concluding where the LOEL/NOEL lies, particularly if this endpoint is to serve as the basis for acute dietary risk assessment.

He then addresses the statement in the draft document that says there were no decreases in body weight gain at 50 mg/kg/day in the range-finding study. He notes that body weight gain was not significantly altered at any dose level, evidently because of the small number of animals employed and the high variability in the body weight data. It is his opinion that these data do not support a conclusion with respect to effects of the test material on doe body weight. In addition, he states that to conclude a single dose as high as 50 mg/kg would not elicit a meaningful biological effect, ChE data over several days would be needed. He cites an article by Kurtz *et al*[9] in which malathion (95% t.a.i.) was administered intraperitoneally to Sprague-Dawley rats at single doses of 0, 25, 50, 100 or 150 mg/kg. Avoidance behavior was significantly impaired 1 hour after injection at doses of 50 mg/kg and above. No clinical signs were observed except one rat at 150

[9]Kurtz, P. J. (1977) Dissociated Behavioral and Cholinesterase Decrements following Malathion Exposure, Toxicol. Appl. Pharmacol. 42, 589-594

mg/kg exhibited tremors within the 24-hour post dosing period. Also within that period, ChE was significantly inhibited at 100 and 150 mg/kg. The study author concluded that low doses of malathion may disrupt behavior without significantly reducing ChE activity.

2) From Attachment 13: Letter from B. Dementi dated July 29, 1998

This document was not cited by Dr. Dementi in his February 28, 2000 letter, however it is an addendum to his July 27, 1998 memorandum (Attachment 12). It refers to copies of letters from Drs. Dourson and Decker in which they responded to additional questions from Dr. Dementi after he reviewed their initial evaluations. A letter dated July 7, 1998 from Dr. Dementi to Dr. Spencer was supplied by Dr. Dementi on March 23, 2000. The follow-up question concerning Issue 2 was whether doses of malathion that inhibit brain ChE would pose a concern for possible behavioral effects at or below such doses. The question was in reference to the Kurtz *et al* (1977) study. Dr. Dementi says Dr. Dourson's response asked if the effect on avoidance behavior was statistically significant. Dr. Dementi clarifies that it was statistically significant, $p<0.02$. He then concludes that consideration of this and other information in the HIARC reference materials and Dr. Dourson's comments in his item 3 of question 2 would indicate some recognition on his part of the need for conducting the developmental neurotoxicity study on malathion. This evaluator notes that the question concerning statistical significance referred to in this letter is not included with Dr. Dourson's responses that are presented in Attachment 1 of the December 22, 1998 HIARC report. Dr. Dementi was contacted about this omission and he responded by email dated March 15, 2000, that the question from Dr. Dourson appears in his (Dourson's) July 17, 1998 response addressed to William Burnam. He then provided this evaluator with a copy of the letter.

Evaluation by External Peer Review Members

The three questions submitted to the panel under I. Hazard Identification/Acute Oral (One-Day) concern Issue #2 of Dr. Dementi's February 28, 2000 letter. In the first question, the panel was asked if the rabbit developmental toxicity and range-finding toxicity studies support a conclusion that a single oral dose of malathion as high as 50 mg/kg would be without toxicological consequence in either maternal or developing organisms. Dr. Decker, answered," *No for reasons cited in the reviews of DERs # 5 and # 9."* However, the DER numbers are not the correct ones for these studies. Under the correct numbers (7 and 19), the developmental toxicity study and range-finding study are both judged to be acceptable; the only additional comment is that the range-finding study is not a definitive treatise on the developmental toxicity of malathion to rabbits. Therefore, Dr. Decker did not answer the question. Dr. Dourson, stated that the available data do not allow this question to be answered. He postulated about the possible dosage range for a No Observed Effect Level (NOEL) based on cholinesterase inhibition (ChEI), which is the critical single day effect, in his opinion. He asked if there are any acute oral studies that test for ChEI. (It is assumed that the acute neurotoxicity study DER, which measures effects after a single dose, was provided to him.) Dr. Hartung said the range-finding study was of limited value but showed no significant effects at 50 mg/kg/day during gestation days 6-18. He judged the developmental study as a more complete study which showed increased resorption sites at 25

mg/kg. [This conflicts with the December 17, 1997 HIARC report (II. Hazard Identification, A. Acute Dietary) which says the developmental NOEL was 25 and the Lowest Observed Effect Level (LOEL) 50 mg/kg/day based on slightly increased incidence of mean resorption sites per dam.] He also says that the available information is inconclusive whether a single dose, administered during a day of maximum sensitivity, would be able to elicit the observed response or whether cumulative dosing is required. (It is assumed by observed response, he refers to the increased incidence of mean resorption sites.)

In his consolidation report (Attachment 12 of February 28, 2000 letter), Dr. Dementi concludes that all three toxicologists responded, "*No.*" In his comment section, he said, "*The external reviewers do not accept that a single dose as high as 50 mg/kg would be without toxicologic effect in maternal or developing organisms based on the rabbit developmental toxicity studies."*

The December 22, 1998 HIARC report summarized the panel's responses as, *"The Panel did not think the Agency's acute dietary endpoint of 50 mg/kg was justified based on the rabbit data and thought that an acute oral study measuring cholinesterase would be better."*

In summary (evaluator's opinion), Dr. Dourson said the available data do not allow this question to be answered. Dr. Hartung said the available information is inconclusive at least for the developmental effect. Therefore, two of the three panel members did not answer the question directly. Although Dr. Decker answered the question no, his reference to the basis for his conclusion was not correct.

The second question for the panel was whether recently available data on maternal body weight and body weight gains alter the assigned LOEL/NOEL for the rabbit developmental study and does it influence the interpretation as to whether a single dose of malathion of 50 mg/kg would be without toxic effect. Dr. Decker answered, "*No, the statistical significance of maternal body weight and body weight gain is not available*." Dr. Dourson said the maternal body weight gain is not relevant to the discussion of the critical effect after acute exposure. He again asked if there are any acute oral studies that test for ChEI. Dr. Hartung said the body weight and body weight gain data can be interpreted as evidence of slight toxicological effects in pregnant does at 50 mg/kg and higher. He continued to say that this evidence of maternal toxicity may be the basis for increase resorption sites at these dose levels. (In the previous question, he said the increased resorption sites were observed at 25 mg/kg.)

In his consolidation report (Attachment 12 of February 28, 2000 letter), Dr. Dementi again concludes that each toxicologist has responded, "*No*." In his comments section, Dr. Dementi says, "*The external reviewers agree that data in Appendix III would not influence the conclusion. We should note that data in this appendix has not been analyzed, statistically, in HED."*
The 1998 HIARC report states the Panel's response as, "*The panel was not influenced by the new data but thought it showed a slight toxic effect at 50 mg/kg, but data are not relevant for a single exposure at this dose."*

In summary (evaluator's opinion), Dr. Decker answered no. Dr. Dourson said body weight gain was not relevant and Dr. Hartung said there was evidence of an effect at 50 mg/kg/day.

The third question concerned a study in the open literature in which a single intraperitoneal dose of 50 mg/kg in the rat reportedly elicited a clear effect on avoidance performance. Erythrocyte ChEI was observed at 100 mg/kg. Plasma and brain ChEI were inhibited at 150 mg/kg. The question was asked in three parts: 1) what level of confidence should be accorded this study; 2) what is the implication of the route of administration to the question of whether a single oral dose of 50 mg/kg can serve as an endpoint for acute dietary (one-day) risk assessment; 3) are the data available in the developmental toxicity studies sufficiently reliable to discount the 10x safety factor required under FQPA. The third part of this question will be addressed with Issue #1 of Dr. Dementi's February 28, 2000 letter.

Dr. Decker responded to the first two parts of the question by saying that the intraperitoneal route of administration cannot be directly compared to dietary administration. Dr. Dourson responded that, while the study tested for what may be the likely relevant effect, the route of exposure is an issue. He proposed that kinetic data suggesting a speed and percent of absorption after oral exposure could be used for a comparison to the intraperitoneal study. (It is assumed that he meant an equivalent oral dose could be calculated from the 50 mg/kg intraperitoneal dose.) Dr. Hartung responded that the use of the intraperitoneal dosing as a surrogate for environmentally relevant routes of exposure cannot be defended quantitatively. He also said that the distribution patterns and pharmacokinetics associated with intraperitoneal dosing are sufficiently different from other routes to render most quantitative association very doubtful and to create reservations even for qualitative assessments.

In his consolidation report (Attachment 12 of February 28, 2000 letter), Dr. Dementi summarized the responses as follows:

"Dr. Dourson: Says the study has advantage of testing a relevant effect. Route of exposure is an issue. "I am not satisfied that potential risks to humans is addressed with the data available in this review package. But more data are probably available to further address this question. A discussion of uncertainty factors for potential data base gaps should be postponed pending the review of these additional data." (p. 4)

Dr. Hartung: Says behavioral effects that have a degree of correspondance with cholinesterase inhibition are to be expected, but there is no requirement that dose response curves for both to coincide. Intraperitoneal route is of questionable surrogacy for realistic exposures. Says data does not support deletion of the 10X factor.

Dr. Decker: Accord low level of confidence to the study because i.p. cannot directly compare to real exposures. Says cannot dismiss the 10X factor."

In his comments, he states, "*The external reviewers consider the study to be of value in that it*

assesses relevant effects, and supports a degree of correspondence between cholinesterase inhibition and behavioral effects, but all appear to agree that data from developmental toxicity studies, and perhaps the entire malathion data base, does not support deletion of the 10X safety factor imposed by FQPA. My principal reason for citing Kurtz (Ref. D) was to illustrate that a single dose at 50 mg/kg can elicit a remarkable response. Furthermore, the study shows that at doses extending below those inhibiting cholinesterase, a behavioral effect has been observed, even if the route of administration differs from that of normal human exposure. None of the reviewers question the quality of the study, or the validity of the findings."

The 1998 HIARC report summarized the panel's findings as, "*One member accorded low level of confidence to the intraperitoneal (i.p) study because i.p cannot be directly compare to relevant real-life exposure scenarios. The second stated that the intraperitoneal route is of questionable surrogacy for realistic environmental exposures. While, the third member reported that the study has the advantage of testing a relevant effect, he also stated that the route of exposure is an issue*."

In summary (evaluator's opinion), all the panel members concluded that the route of administration complicates the use of the study.

December 22, 1998 HIARC Report

Concerning question number 1, the HIARC concluded:

The Committee concluded that based on the combined results of the Range-Finding and Main Rabbit development study, a single oral dose of 50 mg/kg could be estimated to have no toxicological effect (i.e.,NOAEL) and thus is appropriate for acute dietary risk assessment. This dose was selected from a compilation (synthesized) of studies and is considered to be conservative for a single exposure (acute) dietary risk assessment. The rationale for sustaining 50 mg/kg/day as the NOAEL for acute RfD is as follows: In the Range-Finding study no deaths occurred at 100 mg/kg/day. Death attributable to a single dose (i.e., the period of exposure of concern) occurred only in 1 doe on GD7 at 400 mg/kg/day and in does at 200 mg/kg/day after multiple doses (i.e., gestation days 11 and 17). Clinical signs seen in both studies were not attributable to a single dose. In the Main Study, the LOEL of 50 mg/kg/day was based on decrease in mean body weight gains in does during the dosing period. This decrease in mean body weight gains was not attributable to a single dose but rather to multiple doses. It should be noted no mortalities, clinical signs or decreases in body weight gain were seen when the same dose was tested in the Range-Finding study. Thus, toxicological endpoints (e.g., death, clinical signs, or certain developmental abnormalities) attributable to a single dose were not observed in does at 50 mg/kg/day. Also, this dose was selected after review of the other oral studies (which are suitable for use in this risk assessment) that had much higher NOELs/LOELs such as the acute neurotoxicity study in rats (NOEL=1000 mg/kg/day, LOEL = 2000 mg/k) and the developmental toxicity study in rats (maternal NOEL=400 mg/kg/day, LOEL=800 mg/kg/day, developmental NOEL=>800 mg/kg/day). In particular, the acute neurotoxicity study in rats was

not useful since cholinesterase data in this study showed much variation and a poor dose response relationship and thus was not appropriate for a regulatory endpoint.

Concerning question number 2, the HIARC concluded:

The HIARC, again based on the weight-of-evidence of the data base (see rationale above for question 1), reaffirmed its original conclusion that 50 mg/kg/day is appropriate for acute dietary risk assessment.

$$\text{Acute RfD} = \frac{\text{50 mg/kg/day (NOEL)}}{\text{100 (UF)}} = 0.5 \text{ mg/kg/day}$$

Concerning question number 3, the HIARC concluded:

The HIARC considered this route to be not appropriate for acute dietary risk assessment.

Evaluator's Comments on Accuracy, Clarity and Transparency of HIARC Reports Concerning Issue 2

Studies in which the critical endpoint is measured after a single dose should be considered initially in establishing a dose and endpoint for an acute RfD. If available, the study usually selected for the general population (including infants and children) is the acute neurotoxicity study. However, any other study in which effects are demonstrated after a single dose may be applicable for this population. Rat and rabbit developmental studies may be useful because both maternal and fetal effects are measured. To be applicable to the general population, maternal effects must be observed after a single dose. Fetal effects may be used for establishing an acute RfD in females 13-50 if the fetal findings can be assumed to occur after a single dose. If an appropriate study and endpoint attributable to a single dose are not available, an acute RfD for the population (either general or females 13-50) is not established.[10] Therefore, in the case of malathion, the HIARC reports should be clear as to why the acute neurotoxicity (general population) and the developmental studies (general and/or females 13-50) were not useful for the acute dietary risk assessment. If there were no studies in which effects were measured after or attributable to a single dose, the HIARC reports should clearly state why an acute dietary risk assessment was necessary and how repeated dose measurements were appropriate for the acute RfD.

1. Acute Neurotoxicity Study

Some of the discussion in the HIARC reports of December 17, 1997, and December 22, 1998, on why the deficiencies in the acute neurotoxicity study made it inappropriate for endpoint selection are unclear. Both of the reports indicate that there was low confidence in the ChE data. The 1997

[10] HED Risk Assessment Training Manual, Fall, 1998

report states that, in rats given a single oral dose of malathion at 0, 500, 1000 or 2000 mg/kg, plasma and erythrocyte ChE were inhibited in both sexes at 2000 mg/kg on Day 7, a finding which was sustained in females only on Day 15. The implication to this evaluator is that the finding on Day 15 reduces the confidence in the data. This is unclear. It is not unexpected that ChE levels would rebound by 15 days after a single dose. In the discussion of this study in the 1997 report under III. FQPA Considerations, Neurotoxicity Data, it states that on Day 1 of the study, the 2000 mg/kg animals had decreased motor activity and clinical signs (salivation, body staining, one death with tremor, labored breathing, stained fur, decreased defecation and urination). Therefore, clear evidence of toxicity existed at 2000 mg/kg.

The 1997 report says there was equivocal inhibition of plasma ChE in females at 500 and 1000 mg/kg, which was characterized by a poor dose response. The report does not say when the inhibition was observed post dosing and if it was statistically significant affected at either dose. (It is assumed that the response was not statistically significant since it was characterized as an equivocal inhibition.) The report also indicates there was lack of a dose response and a clear NOEL for this biomarker since inhibition of ChE activity was seen in other studies among various species. It is unclear if inhibition was seen after a single dose in the other studies.

The 1998 report repeats much of the information in the 1997 report. It states that the 50 mg/kg dose was selected for the acute risk assessment after review of other oral studies that had much higher NOELs/LOELs, such as the acute neurotoxicity study in rats (NOEL=1000 mg/kg; LOEL=2000 mg/kg). It further states that the acute neurotoxicity study was not useful since ChE data showed much variation and a poor dose response relationship and thus was not appropriate for a regulatory endpoint.

In summary, more detail about the ChE dose response relationship, e.g., which compartment was affected at 500 and 1000 mg/kg, what level of inhibition was observed, would help to explain why the study was not useful and explain the basis for rejecting this study in favor of a repeated dose study for the acute dietary risk assessment.

2. Developmental Studies

a. Developmental Endpoint - Rabbit Study

The 1997 report states that the increase in resorption sites/dam at 50 mg/kg was not considered to be an appropriate endpoint because the incidence was only slightly increased and was considered by the Committee to be of no meaningful toxicological significance with respect to acute dietary risk assessment. (The obvious question to this evaluator is if this increase was of no toxicological significance, why was it considered a LOEL.) The 1998 report does not discuss this endpoint *per se* but states that the toxicological endpoints (e.g., death, clinical signs or certain developmental abnormalities) attributable to a single oral dose were not observed in does at 50 mg/kg/day. (It is noted that developmental effects are observed in fetal animals.) The reports are unclear about why the slight increased incidence in resorption sites was not adequate for risk assessment. As noted previously, one of the external peer review toxicologists thought there was an increase in resorption sites at 25 mg/kg/day. More information on statistical significance and dose response would help clarify why this endpoint was not selected.

b. Maternal Endpoint - Rabbit Study

It is unclear from both the 1997 and 1998 reports exactly what maternal endpoint was used for the acute risk assessment. Under Dose and Endpoint for Risk Assessment in the 1997 report, it gives a NOEL of 50 mg/kg with no endpoint stated. In the Summary of Toxicology Endpoint Selection table, the endpoint for acute dietary is listed as "maternal toxicity". This table is repeated in the 1998 report. The rationale for selecting 50 mg/kg as the NOEL for the acute dietary risk assessment is very circuitous.

c. Maternal and Developmental Endpoints - Rat Study

The 1998 report explains that the developmental study in rats was not used because the NOELs/LOELs were higher than the 50 mg/kg selected.

<u>Evaluator's Comments on HIARC Reports Reflecting Dr. Dementi's Opinions on Issue 2</u>

1. One point raised by Dr. Dementi was not adequately addressed in the reports. Apparently, individual animal body weight data were submitted for maternal animals in the developmental rabbit study after the 1997 HIARC report was issued. In Attachment 6, letter dated December 17, 1997, Dr. Dementi questions if these data have been reviewed. In his consolidation of the peer review panel's responses (Attachment 12 of his February 28, 2000 letter), he states that the data have not been statistically analyzed. The record should reflect if the data have been reviewed and evaluated by the Agency.

2. Dr. Dementi's February 28, 2000 letter indicates that the acute RfD is unsupportable because of an absence of ChE measurements in the developmental studies. He says the external peer review

panel agreed with this position. This evaluator could not find a specific question about ChE measurements in the developmental studies. The third part of question 3 asked if the data available in the developmental studies are sufficiently reliable to discount the 10x safety factor but didn't specifically ask about ChE measurements.

ISSUE 3: Hazard Identification/Chronic Dietary (RfD)

*The HIARC established the Chronic RfD based upon cholinesterase inhibition as derived from the combined chronic toxicity/carcinogenicity study in the rat. This decision was rendered though two members of the External Peer Review Panel, in addition to myself, affirmed retention of the human study as the basis for the RfD, while the third panel member, though supporting the rat study, advocated an additional 3-fold uncertainty factor be applied to address study deficiencies in the rat ["...., principally because the critical effect was not monitored in the 2 generation reproductive study in a potentially sensitive subgroup [i.e., young rats (emphasis added)]." (Dourson, p. 30) Dr. Dourson also advocated an additional 3-fold safety factor be applied to the human study derived RfD, should it be retained: "The use of the human data has the obvious advantage of relevance. However, it does not test females, so the NOEL/LOEL range could potentially be lower. The use of the data base factor of 3-fold would also lower the RfD." (p. 30) So the HIARC has disregarded the recommendations of the entire External Review Panel; as well as my recommendation, which was to retain the human study (with an added uncertainty factor to compensate the absence of cholinesterase data in women), while conducting a more definitive assessment of cholinesterase inhibition in the rat. However, I am also enamored of Dr. Dourson's expressed concerns over the absence of cholinesterase data in young rats, which applies, I might add, to the human data as well, as being consonant with FQPA concerns. In retaining its decision, the HIARC has not specifically addressed the rationale of Panel members nor myself. The Panel had much to say, the content of which may be found in their appended responses (**Att 1**) and is summarized in my July 21, 1998 memorandum (**Att 12**)*

Cited in Dr. Dementi's February 28, 2000 Letter: *:* **057701ha.002:** pp. 8-9; **Att** 1; Att **2:** 52-53, 74-88; **Att 3**; **Att 4:** pp. 103-104; **Att 5**; **Att 6:** p. 110; **Att 12:** pp. 127-129; **Att 18:** pp. 149-150.

Additional Information from Dr. Dementi's Detailed Memoranda/Letters

1) From Attachment 3: Letter from B. Dementi dated November 10, 1997

This letter, along with four exhibits, is a follow-up to the November 6, 1997 HIARC meeting. Dr. Dementi expresses his concern that the HIARC shifted the basis for the chronic RfD from the NOEL in the human study (Moeller and Rider, 1962) to the NOEL for ChEI in the 1996 rat combined chronic toxicity/carcinogenicity study. He thinks this decision was too precipitous, in that it should have been presented as an issue or topic well before the meeting to allow for better preparation. He states that, based on the valid assessment of the LOEL/NOEL for ChEI in human subjects, evidence suggests that humans are at least 10-fold more sensitive than rats for RBC ChEI and even more sensitive for plasma ChEI. He says that, during the discussion at the HIARC meeting, someone said the shift in purity between the 1962 vintage malathion and the 1996 product could explain the species differences. But, he says, humans have been historically more sensitive, i.e., were more sensitive than the rat on the basis of earlier products and he speculates

are likely to remain so based on the new product. He postulates that the reason for the difference is the level of carboxylesterase activity. This enzyme, "*...via catalysis of (sic) hydrolyis of one carboxyethyl group on malathion (actually malaoxon as the cholinesterase inhibiting entity) compromises its cholinesterase inhibiting capabilities.*" He indicates he stated at the HIARC meeting that insects lack carboxylesterase activity, which is thought to explain malathion's insecticidal efficacy. He says that published works show carboxylesterases are located in the plasma and liver of the rat, but are only found in the liver of humans. Exhibit 1 (*Comments on the Potential Role of Aliesterases in Malathion Toxicological Assessments*) to this letter contains a review of the literature on the various carboxylesterases in the plasma and tissues of animals. It will not be discussed in detail in this document.

Concerning the more purified malathion used in the rat study, Dr. Dementi states that there should be more concern because ChE inhibiting impurities (malaoxon and isomalathion) were reduced compared to the 1962 human study. But before the HIARC accepts the claim about the differences in purity to explain the species differences, the Confidential Statement of Formula should be compared for the respective products. He indicates that he has compared the two products, and the levels of malaoxon and isomalathion are reduced in the new product. However, he questions the relative effects of these entities at low doses where metabolic conversion of malathion to malaoxon is less saturated.

Regarding the combined chronic toxicity/carcinogenicity study, he says the registrant was advised that 100 ppm would likely not be the NOEL for the study based on blood ChEI. Exhibit 2 to the letter contains a memorandum of the December 10, 1991 meeting with the registrant, which verifies this recommendation.

After three months into the combined chronic toxicity/carcinogenicity study, there was a statistically significant decrease in RBC ChE in females at 100 ppm so the dose was reduced to 50 ppm, which proved to be the NOEL for RBC ChEI in both sexes. Exhibit 3 contains a page from the combined chronic toxicity/carcinogenicity study which Dr. Dementi thinks indicates that the effect at 100 ppm corroborated the findings in the Sprague-Dawley rat performed 17 years ago with the old malathion product. He concludes that there is little improvement in the new product with respect to RBC ChEI at low doses, particularly those critical to setting the RfD for malathion. Additional ChE information is called for in view of the absence of a NOEL among females at the 3-month time point. There is no assurance that the enzyme would not have been inhibited at 50 ppm during the first three months. Exhibit 4 contains ChE data from the combined chronic toxicity/carcinogenicity study.

He continues that this is very important in view of the facts that:

a) malathion has a very shallow dose response curve. In his judgment, there is little difference between 50 and 100 ppm for an agent that demonstrates such a shallow dose response curve ranging up to 6000-12000 ppm.

b) the human study demonstrated greater sensitivity for uncertain reasons.

c) the number of animals (10/sex) assayed for ChE activity does not give sufficient statistical power to clearly identify a NOEL at low but meaningful levels of inhibition.

Therefore, he reiterates his point that a definitive NOEL must be determined using large numbers of rats at doses that embrace those used in the human study overlapping those of the lower dose range in the rat chronic toxicity/carcinogenicity study, say up to 20 mg/kg/day.

2) From Attachment 4: Letter from B. Dementi dated November 20, 1997

This letter concerns Dr. Dementi's position on the conclusions of the November 13, 1997 ad hoc neurotoxicity subgroup meeting. Concerning Issue 3, he states that the subgroup agreed that sex-related differences are manifest in the malathion data base but did not concur that the differences merited a "correction" factor applied to the chronic RfD. He says that, if the human study is used for the RfD, data obtained from men only would be used to protect the entire population. He maintains that a larger safety factor than 10 should be used since female laboratory animals are more sensitive than males.

3) From Attachment 5: Letter from B. Dementi dated November 25, 1997

This letter comments further on the chronic RfD for malathion. Dr. Dementi restates his position that the ChE data from the rat chronic toxicity/carcinogenicity study are inadequate to define a NOEL for female rats. Therefore, a data gap exists and hence, proper data do not exist in this study for the RfD. Much of the contents of the letter deal with the issue of female sensitivity and are summarized under this attachment for Issue 7.

4) From Attachment 6: Letter from B. Dementi dated December 17, 1997

This letter comments on the December 4, 1997 draft HIARC report. Under B. Chronic Dietary [Reference Dose (RfD)], Dr. Dementi reiterates his position about the lack of a NOEL for ChEI in females for the first three months of the rat chronic toxicity/carcinogenicity study. Many of his arguments are repeats of those made in Attachments 3, 4 and 5. In addition, he says the fact that RBC ChE was inhibited in female rats at 100 ppm and 500 ppm at the three month time point but not at the 50 ppm and 500 ppm dose levels at the six month time point is inexplicable. Possible explanations are that there is adaptive recovery post three months (in which case 50 ppm is not a definitive NOEL for the three month time period) and too few animals were employed to obtain good ChE data in view of the shallow dose response for malathion. These possible explanations support conducting a definitive ChE assessment over a three month time period with an adequate number of animals for statistical analyses. Another possible explanation is flawed ChE methodology. Dr. Dementi believes that until a NOEL for ChE inhibition among females has been established, the transfer of the RfD from the human study to the rat study lacks adequate support.

5) From Attachment 18: Letter from B. Dementi dated November 5, 1998

This letter comments on the November 5, 1998 draft report of the August 1998 HIARC meetings. Dr. Dementi's comments on the HIARC summary of the panel's responses. (The responses to all six questions are summarized together in the December 22, 1998 HIARC report.) He says it should be identified that the critical effect in Dr. Dourson's quote is ChEI. He then states that the other panel members should also be quoted. He suggests the following quotes from Dr. Hartung, ***"No. The human is the correct species of concern. Substituting a rodent introduces many more uncertainties than those produced by minor deficits in the analysis of chemical purity or concern about statistical precision."*** *(p. 7 of his 6/3/98 comments); and* ***"Look at what you are doing! Here you are willing to accept a study for which you are also willing to mess around with another factor of 10X, just because the statistical data are neater. In the process you are willing to discount human data, even though it is extremely unlikely that the equivalent statistical uncertainties for the human will reach anywhere close to 10X."*** *(p. 8 of his 6/3/98 comments)."* (See discussion below for responses to individual questions.) Dr. Dementi then discusses the purity issue. He states that, although purity was not given the Moeller and Rider human study, the American Cyanamid product was used and its purity was known at the time of the study. He indicates that there was discussion at the HIARC meetings that the rat was a poor surrogate for the human because of carboxylesterase differences. He says the HIARC concluded on August 18, 1998, to impose an additional UF but the decision was reversed on August 20, 1998 because the issue was not addressed with other pesticides. He says this aspect of the deliberation "*...finds no entry in these draft minutes*." He then quotes Dr. Decker as saying, "***Additional testing should be required in the male <u>and</u> female rat before any thought is given to replacing the human data relied on to establish a RfD."*** *(p. 5 of his 6/11/98 comments)* Dr. Dementi thinks the following section should be added to the 1998 HIARC report, "*In summary, two external reviewers were firm in recommending against switching to the rat study, while the third member favored the rat study, contingent upon imposition of an additional 3-fold uncertainty factor. The committee is ignorant as to the latter's views regarding the use of the rat versus the human study in the absence of an imposed additional uncertainty factor.*"

<u>Evaluation by the External Peer Review Panel</u>

There were six questions concerning Issue 3. The <u>first question</u> asked if 50 ppm can be concluded to be the NOEL for the first three months of testing, given the evidence of a recovery of RBC ChE in females after three months in the combined chronic toxicity/carcinogenicity study. Dr. Decker said no because the NOEL for RBC ChE was not identified during the first three months. Dr. Dourson said yes, 50 ppm is a NOEL. He said the statistically significant effects on RBC ChE in 100 ppm females at three months could be due to: 1) chance; 2) the female control values were unusually high (>50% higher than value at six months and even higher when compared to two male control values at three and six months); 3) fact that the administered dose was 100 ppm in the diet for the first three months (most likely explanation). Since animals would eat more diet on a body weight basis when they are younger (i.e., three months), then when they were older (i.e., six months), their dose after three months at 100 ppm would be expected to be more than the

difference between 100 ppm and 50 ppm at six months. Dr. Hartung said, *" No. Once the malathoxon-acetylcholinesterase bond is established in RBCs, the rate of recovery is identical to the red cell replacement rate, which is approximately 1%/day."*

In his consolidation report (Attachment 12 of February 28, 2000 letter), Dr. Dementi summarized the panel's responses as follows:

"Dr. Dourson: Yes, but recommends an additional 3-fold uncertainty factor be applied to the NOEL in the rat in establishing the new RfD , as indicated in question 5.

Dr. Hartung: No.

Dr. Decker: No."

In his comments section, Dr. Dementi says, *"Dr. Dourson says yes to this question, but it is not clear what his opinion would be in the event an additional uncertainty factor were not used with the rat data as he proposes. The other two reviewers agree that it cannot be said that 50 ppm was a NOEL in view of the findings in the background papers. Elsewhere in their comments, Dr. Hartung says: "I find the discussion regarding the selection of plasma cholinesterase inhibition for the determination of the RfD to be simplistic and superficial." (p. 3) Dr. Decker says with regard to the question of whether the human or rat data should be used for establishing the RfD: "I recommend that Dr. Dementi's suggestions be actively pursued, that is more studies are needed to fill in data gaps." (p. 4) Dr. Decker thus acknowledges data gaps. He also says: "I am not aware of supporting studies which shore up the use of the principal study for the RfD." (p. 4) It is reasonable therefore to conclude that a consesus exists that the study does not satisfactorily identify a NOEL for cholinesterase inhibition. It should be noted that the registrant was advised before conducting the chronic toxicity/carcinogenicity study in the rat that 100 ppm would be expected to be an effect level for cholinesterase inhibition (Ref. I) Three months is an important time period, as within this time frame important adjustments to the treatment may occur."*

In summary (evaluator's opinion), Drs. Decker and Hartung answered no. Dr. Dourson answered yes.

The second question asked if the rat combined chronic toxicity/carcinogenicity study findings suggest flawed ChE methodology and if so, what corrective measure could be pursued. Dr. Decker said flawed methodology is possible but if this were true, all OP pesticides would give erroneous results. Dr. Dourson said he had no comment on the methodology but that the study seemed well designed and executed. The change in dose levels from 100 to 50 ppm at 3 months seemed reasonable. Dr. Hartung said this requires an analysis of detailed ChE methodology. He asked several questions about the procedure and then cited a reference.

In his consolidation report (Attachment 12 of February 28, 2000 letter), Dr. Dementi summarized

the panel's responses as follows:

"Dr. Dourson: No comment on cholinesterase methodology.

Dr. Hartung: Says requires analysis of detailed cholinesterase methodology.

Dr. Decker: Says this is a possibility, and if so, concern extends to all OP pesticides."

In his comments section, Dr. Dementi concludes that, in the views of the external reviewers, this appears to be a question that requires resolution.

In summary (evaluator's opinion), none of the panelists provided an answer to the question.

The third question asked if the NOEL of 4 mg/kg/day (NOEL for plasma ChEI in males) can be supported as a replacement for human data or should additional testing be required in the rat to identify a NOEL for ChEI, particularly in females. Dr. Decker said additional testing should be required in male and female rats before replacing the human data for the RfD. Dr. Dourson said additional testing is not needed; the 50 ppm dose is the NOEL in females for the study. He continued that if some want to pursue whether 50 ppm is a NOEL for females, a benchmark dose analysis could be done on the existing female responses or the results of the 2 year study could be compared to the subchronic neurotoxicity study, where there was a similar dose range and similar results were found. The low dose of 50 ppm in the subchronic neurotoxicity study was a NOAEL in both males and females. (The use of NOAEL, instead of NOEL is not explained.) Dr. Hartung said the human is the correct species of concern. Substituting a rodent introduces many more uncertainties than those produced by minor deficits in the analysis of chemical purity or concerns about statistical precision.

In his consolidation report (Attachment 12 of February 28, 2000 letter), Dr. Dementi summarized the panel's responses as follows:

"Dr. Dourson: Yes to the first part of question. Says additional testing not needed. Suggests benchmark dose analysis in event some scientists wish to pursue whether 50 ppm is a NOEL in females. Notes that 50 ppm was a NOAEL in the 13-week neurotoxicity study. However, recommends additional 3-fold uncertainty factor as indicated in Question 5.

Dr. Hartung: No to the first part of question, and is critical about replacing human data with animal data.

Dr. Decker: No to the first part of question. Recommends additional testing to identify NOEL in rats of both sexes."

In his comments section, Dr. Dementi refers to comments under question 1. In addition, he states he has concerns about relying on the NOEL for ChEI in the subchronic neurotoxicity study, as

expressed in his December 17, 1997 letter.

In summary (evaluator's opinion), Dr. Decker said additional testing in rodents is required. Dr. Dourson said additional testing was not needed. Dr. Hartung said the human is the correct species.

The fourth question asks if a 10x safety factor applied to the rat data to allow for "uncertainties" in interspecies variability should be considered adequate if the rat data are used for deriving the RfD, given that an explanation exists for a greater sensitivity of humans than rats to malathion ChEI (i.e., lack of carboxylesterase in human plasma). Dr. Decker said he thought the 10x safety factor was reasonable if enhanced rat data (from additional testing recommended in question 3) were used in deriving the RfD. Dr. Dourson did a crude comparison of the NOEL/LOEL between the human and rat studies. Using the male human values of 0.23 and 0.34 and the rat values of 4 and 29 mg/kg/day, the NOELs are approximately 20-fold apart. The LOELs are further but he says this likely reflects the fact that the rat NOEL and LOEL are further apart than necessary in the rat study. (Assumed to mean due to dose levels.) Furthermore, the human study was by gavage and the rat by feeding. Gavage dosing may likely lower the NOEL as compared to a feeding study. It is his judgment that a 10-fold UF is necessary and satisfactory. No additional factor is needed. Dr. Hartung said, *"Look at what you are doing! Here you are willing to accept a study for which you are also willing to mess around with another factor of 10X, just because the statistical data are neater. In the process you are willing to discount human data, even though it is extremely unlikely that the equivalent statistical uncertainties for the human will reach anywhere close to 10X !"*

In his consolidation report (Attachment 12 of February 28, 2000 letter), Dr. Dementi summarized the panel's responses as follows:

"Dr. Dourson: Yes, but advocates an additional 3-fold uncertainty factor for other reasons as indicated in question 5.

Dr. Hartung: No

Dr. Decker: No, but would be acceptable with enhanced testing in the rat."

In his comments section, Dr. Dementi says, *"The reviewers' comments are important in underscoring the fact that the data base is inadequate as it stands in establishing an RfD. Actually, in posing this question, I was seeking the reviewers' opinions as to whether the concept of using a 10-fold safety factor intended to account for uncertainties in interspecies variability is adequate in the face of known differences in sensitivity. Stated differently, should corrections to accomodate know differences, which may even exceed 10-fold, first be introduced, followed by the 10-fold factor to address the unknown species differences in susceptability? (Ref. I) It is not clear to me that this particular philosophical question was recognized or responded to, but remains a question for the Hazard ID Committee."*

In summary (evaluator's opinion), Drs. Decker and Dourson said no additional UF is needed for interspecies extrapolation. Dr. Hartung did not answer the question directly.

The fifth question asked if the RfD should be based on human data, given that the RfD based on the human study (0.023 mg/kg/day) is lower than that derived from the rat study (0.040 mg/kg/day). Dr. Decker said the RfD based on human data should be retained for the present time because humans are apparently more sensitive. Dr. Dourson said the rat study appears to be a stronger basis for the RfD. He thinks the rat study NOEL should be divided by a 3-fold UF for deficiencies in the data base, particularly because the critical effect was not monitored in potentially sensitive subgroups in the reproduction study. He then postulates whether the 3-fold data base factor should be used for the human study since it did not test females. Dr. Hartung simply said yes.

In his consolidation report (Attachment 12 of February 28, 2000 letter), Dr. Dementi summarized the panel's responses as follows:

"Dr. Dourson: No, but advocates an additional 3-fold uncertainty factor to account for deficiencies in the data base, principally because the critical effect (cholinesterase inhibition) was not monitored in the 2-generation reproduction study in a potentially sensitive subgroup (i.e. young rats), which he characterizes as a data gap (p. 3). Also, suggests an added uncertainty factor of unspecified magnitude, probably less than 3 in his view, for the RfD based on the human study, should it be retained, since females (women) were not tested.

Dr. Hartung: Yes.

Dr. Decker: Yes."

In his comments section, Dr. Dementi says, *"Given that Drs. Hartung and Decker say, emphatically, the human study should be retained, and Dr Dourson does not provided an unqualified differing opinion, a consesus exists that the human study should be retained. If it is to be retained, an added safety factor should be considered based upon Dr. Dourson's comments."*

In summary (evaluator's opinion), Drs. Decker and Hartung recommended using the human study for the chronic RfD, whereas Dr. Dourson recommended using the rat study.

The sixth question asked whether this study (assumed to be the rat combined chronic toxicity/carcinogenicity study) provides any support for discounting the 10x safety factor, other than contributing to the completeness of the data base. Dr. Decker said no because infants and children are likely to need additional protection since they, in general, are more subject to toxic insult than adults. Dr. Dourson said he doesn't think the FQPA safety factor should be considered in a discussion of the science behind whether a data base UF should or shouldn't be used. He then presented excerpts from public comments he gave on FQPA. He provided his philosophy on

whether an additional safety factor is needed for children and at what part of the risk assessment/risk management process it should be applied. Dr. Hartung referred to his previous discussion related to 10x FQPA safety factor but did not identify specific responses.

In his consolidation report (Attachment 12 of February 28, 2000 letter), Dr. Dementi summarized the panel's responses as follows:

"Dr. Dourson: Does not answer the question as such, but acknowledges in Question IV, # 5 recognition the study does not test toxicity in young rats, and, hence, lacks surrogacy for infants and children. He asserts that the FQPA safety factor should not be considered in a discussion of science. He discusses his interpretation of the FQPA 10X factor as a safety factor for use in risk management toward the protection of infants and children, as opposed to that of an uncertainty factor.

Dr. Hartung: No, since the available information does not support the hypothesis that neonates are less sensitive than adults (see his p. 6)

Dr. Decker: No."

In his comments section, Dr. Dementi says, "*In disagreeing with the context of the use of the 10X safety factor, Dr. Dourson in my view did not respond with an opinion as to whether this study in any way supports discounting imposing the factor. Drs. Hartung and Decker say no. It would appear reasonable to conclude the reviewers feel the study does not provide any support for discounting use of the safety factor."*

In summary (evaluator's opinion), Dr. Decker said no. Drs. Dourson and Hartung didn't answer the question directly. Dr. Hartung didn't identify the comments to which he referred. The page 6 cited by Dr. Dementi is taken out of context from the answer to question 3 of Issue 1 concerning the developmental rabbit study. In addition, Dr. Dementi's interpretation of Dr. Dourson's response is confusing. The response he refers to in Issue IV, question 5, concerns the subchronic inhalation study.

The December 22, 1998 HIARC report summarized the panel's responses as follows:

"*In their responses to these six questions, the panel made several assertions, suggestions and recommendations with regard to: (I) establishing a NOEL for the first three months in the two-year rat study (Question # 1); (ii) the adequacy of the cholinesterase methodology (Question # 2); (iii) the need for additional testing to identify a NOEL for cholinesterase inhibition (Question # 3); (iv) the need for additional uncertainty factors to account for deficiencies (Question # 4); and (v) the discounting of the 10x factor (Question # 6).*

With regard to question #5 whether the human study should be retained for deriving the RfD, two members said yes, the human study should be retained since human is the correct species of

concern while the third member said no, "the rat study appears to be a stronger basis for RfD than human work" but advocated "a 3-fold uncertainty factor to account for deficiencies in the database, principally because the critical effect was not monitored in the two-generation reproduction study in a potentially sensitive subgroup (i.e., young rats)". This member also suggested that should the human study be retained, an additional uncertainty factor of "unspecified magnitude, probably less than 3, be applied" since human females were not tested."

December 22, 1998 HIARC Report

Concerning the chronic RfD, the HIARC concluded**:**

"The HIARC reaffirmed its decision to derive the chronic RfD based on the NOEL of 4 mg/kg/day established in the combined chronic toxicity /carcinogenicity study in rats and the use of a UF of 100 to account for inter-species extrapolation and intra-species variation. The RfD remains at 0.04 mg/kg/day.

The HIARC concluded that the human study is not appropriate based on the following factors: (I) there is the low confidence in the human study because of possible confounding factors (e.g., smoking), the purity of malathion is unknown, and the raw data is unavailable for proper evaluation (published in 1962 in open literature); (ii) purity of malathion tested in the animal study is known (97.1%); (iii) the NOEL in the two-year rat study is supported by the NOEL of 4 mg/kg/day established in the subchronic neurotoxicity rat study (based on inhibition of cholinesterase activity); and (iv) the animal toxicology data base is complete except for the subchronic feeding study in dogs and an subchronic inhalation toxicity study in rats.

The HIARC also concluded that an no additional uncertainty factors are necessary since: (I) a NOEL (not a LOEL) was used to derive the RfD; (ii) this NOEL is supported by the same NOEL in the subchronic study in the same species (rats) for the same effects (cholinesterase inhibition) indicating no cumulative toxicity response over time; (iii) the RfD of 0.04 mg/kg/day derived using an animal study with a UF of 100 (for inter-species extrapolation and intra-species variation) is comparable to the RfD of 0.02 mg/kg/day that can be derived by the use of the NOEL of 0.23 mg/kg/day from a human study and a UF of 10x for intra-species variation."

Evaluator's Comments on Accuracy, Clarity and Transparency of HIARC Reports Concerning Issue 3

1. It appears that the human study was re-evaluated during the August 1998 HIARC meetings as evidenced by the addition of a new reason for not using the human study in the December 22, 1998 HIARC report, i.e., possible confounding factors (e.g., smoking). If the study was re-evaluated in light of the peer review panel's responses, the HIARC report should state this.

2. The current EPA policy is that human toxicity studies will not be used to derive endpoints for risk assessments on the basis of ethical considerations. The Scientific Advisory Panel has been

consulted about this issue on two occasions; the Panel's final report is expected in the near future. However, the decision to not use the malathion human study was made before the broader policy issue concerning the acceptability of human studies had been raised. The HIARC's choice was based on the technical limitations of the 1966 study, rather than on ethical or policy grounds. Because the decision predated the general concerns about human testing, malathion was not included among the cases for which the RfDs had been based on human studies when the HIARC reconsidered them. The external peer review panel was clearly divided on whether or not the human study is preferable to the animal study in deriving the chronic RfD, as discussed under question 5 above. EPA has committed to not base any final regulatory decisions under FQPA on human systemic toxicity studies until there is policy in place that can ensure any studies meet the highest scientific and ethical standards. Considering that the malathion study would have to pass more critical technical scrutiny than it did in 1997 when it was rejected by the HIARC, it is certainly questionable if this study will be used in the future for risk assessment.[11]

3. The questions and responses concerning an additional UF are confusing. Part of the problem is that the HED Standard Operating Procedures for determining the FQPA safety factor have changed since the December 17, 1997 report was written. Then, the FQPA 10x safety factor was addressed by the HIARC and discussed in the hazard identification section of the report. Presently, the HIARC evaluates a chemical's potential for increased susceptibility of infants and children. The FQPA Safety Factor Committee considers 1) the contribution of hazard and dose response evaluations in determining whether the FQPA safety factor should be retained, reduced, or removed; 2) the contribution of exposure assessment(s) in evaluating whether retention, reduction, or removal of the safety factor is appropriate; and 3) the characterization of the hazard (toxicology data base) and exposure (dietary food, dietary drinking water, and residential) data bases.[12]

In his response to question 3, Dr. Dourson does say that he thinks the rat NOEL should be divided by a 3-fold UF to account for deficiencies in the data base, principally the reproduction study where the critical effect was not monitored in a potentially sensitive subgroup. However, under question 4 concerning the rat combined chronic toxicity/carcinogenicity study, he says no additional factor is needed. It appears that his remarks concerning the reproduction study deficiencies are more applicable to Issue 1, Food Quality Protection Act (FQPA) 10X Safety Factor for Protection of Infants and Children.

4. The 1998 HIARC report is not clear on whether the six individual questions and the panel's responses were discussed at the August 1998 meetings since there is one summary for all the questions.

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[11] Consulted with John Carley, Office of the Director, OPP, the OPP lead on the human testing issue about EPA policy.

[12] From *Standard Operating Procedures for the HED FQPA Safety Factor Committee*

Evaluator's Comments on HIARC Reports Reflecting Dr. Dementi's Opinions on Issue 3

1. In his February 28, 2000 letter, Dr. Dementi's major concern is about the HIARC's decision to use the rat study instead of the human study to derive the chronic RfD. As discussed above, this issue is in abeyance until EPA receives the SAP recommendations. In his letter, Dr. Dementi also states he thinks a 3-fold UF should be applied if the rat study is used. The December 22, 1998 HIARC clearly states why no additional UF is necessary.

2. The issue of carboxylesterase and whether this can account for species differences in malathion toxicity was not addressed in the December 22, 1998 HIARC report. However, it was not a direct question to the external peer review panel, but was stated as a given fact for question 4.

3. Concerning Dr. Dementi's recommendation that a 3-month study be done to identify the ChEI NOEL in female rats, the HIARC report does not explain why this is not necessary. If there are findings from other studies with similar doses and effects to justify the assumption that the ChEI NOEL was 50 ppm after 3 months, the data should be cited.

ISSUE 4: Subchronic Inhalation Study

Dr. Dementi's Position as Summarized in February 28, 2000 Letter

*At the HIARC meeting of November 6, 1997, the Committee imposed an additional UF of 3 for the intermediate and long term, but not the short term exposure risk assessments. Initially, I disagreed with the use of only a 3-fold UF. Subsequent to receipt of the two-week dose range-finding inhalation study and results of the External Peer Review, the HIARC revised the UF to 10, and directed it be applied to all three time frame risk assessments. The application to short term exposure risk assessments was the result of the finding of nasal histopathology after only two weeks exposure as revealed in the range-finding study. I am concerned as to just how soon following malathion exposure by the inhalation route, effects on the nasal mucosa would be seen, and that HIARC affirm the importance of determining this endpoint in the new inhalation study being required by HIARC. The final HIARC report leaves unaddressed the question of whether a carcinogenicity study by the inhalation route should be performed. (p. 11) In addressing the comments of the External Peer Review Panel, I do not agree with the presentation as set forth in the final HIARC report. My assessment of the responses of the Panel, as presented in my November 5, 1998 comments on the October 27, 1998 draft HIARC report (**Att 18**, p. 150) should have been addressed in the final HIARC report. (pp. 10-11)*

Cited in Dr. Dementi's Letter: **057701ha.002:** pp. 10-11; **Att 2:** pp. 56-58; **Att 6:** pp. 111-112; **Att 9**; **Att 10**; **Att 12:** pp. 129-131; **Att 18**, pp. 150-152.

Additional Information from Dr. Dementi's Detailed Memoranda/Letters

1) From Attachment 6, Letter from B. Dementi dated December 17, 1997

This letter comments on the December 4, 1997 draft report of the November 6, 1997 HIARC meeting. Concerning Issue 4, addressed in the letter under C. Occupational/Residential Exposure, 5. Inhalation Exposure (any-time period), Dr. Dementi states that there was no NOEL in the subchronic inhalation study. Hyperplasia of the olfactory epithelium was locally extensive and that of the olfactory/respiratory epithelial junction was severely affected in all animals. He thinks it is a burden for a study without a NOEL for ChEI and nasal hyperplasia to be used for the short, intermediate and chronic inhalation risk assessments. It is his opinion that a new inhalation study should be required to identify a NOEL for histopathology of nasal tissues. He cites effects of malathion on the olfactory epithelium in the combined chronic toxicity/carcinogenicity study. He then states that the sensitivity of this tissue to malathion rests with its remarkable metabolic capacity, as well as the structure of malathion, a diester of a dicarboxylic acid, which may be hydrolyzed in the olfactory epithelium to yield carboxylic acid. He considers the application of an UF of 3 for the lack of a NOEL as inadequate because of the "*smallness*" of the factor and "*...an operating philosophy which in lieu of weighing the significance of the finding, simply invokes a UF without offering any explanation as to why 3 is adequate, or why another study should not be required.*" Later, in this letter under VI. Data Gaps, he recommends that a longer term study to

address the absence of a NOEL and potential carcinogenicity by the inhalation route be required.

2) From Attachment 9: Letter from B. Dementi dated March 10, 1998

This is an addendum to the December 17, 1997 letter. Dr. Dementi states that he recently reviewed a range-finding inhalation study with malathion, "A 2-Week Toxicity Study of Aerosolized Malathion Administered by Whole-body Inhalation Exposure to the Albino Rat" . Concentrations in the study were 0, 0.56, 1.58 and 4.23 mg/L, as contrasted with the subchronic inhalation study where the doses were 0, 0.1, 0.45 and 2.01 mg/L. After two weeks of treatment, the study report claims that histological findings in the nasal and laryngeal mucosa were observed in most low dose animals and in the majority of mid and high dose animals. He states that the fact there was no NOEL for nasal and laryngeal effects after two weeks of exposure demonstrates a much earlier onset of nasal effects than could be determined from the subchronic inhalation study with malathion and the chronic feeding studies with malathion and malaxon where similar nasal and laryngeal effects were observed. Dr. Dementi argues that the findings of this 2 week range-finding study reinforce his opinion that an uncertainty factor of 3 (for short-term inhalation exposure) is inadequate to compensate for an absent NOEL. He then cites the February 1997 Guidance Document for the Toxicology Endpoint Selection Process and claims that this guideline was not followed, in that the nasal findings were not of negligible concern for human risk, criteria for using an UF of 3.

3) From Attachment 10: Letter from B. Dementi dated March 16, 1998

This is another addendum to the December 17, 1997 letter. The comments concern the 2 week range-finding inhalation study cited in his March 10, 1998 memorandum. He asserts that, in this study, at doses of 0, 0.56, 1.58 and 4.23 mg/L, a NOEL was not identified for red blood cell (RBC) ChEI in either sex or for plasma or brain ChEI in females. He argues that data after two weeks of exposure is complementary to that after 90 days of exposure in the subchronic inhalation study, which demonstrates that there is little evidence of a cumulative effect of malathion over 13 weeks. Dr. Dementi presents the plasma, RBC and brain ChE data for males and females from this study and the 90-day inhalation study in a table. He concludes that the two studies together indicate that RBC ChE is equally responsive in both sexes but that females are more remarkably affected in terms of plasma and brain ChEI. He states that the range-finding data strengthen the conclusion in the subchronic study that there is no NOEL for plasma ChEI in females and possibly for RBC ChEI in both sexes. (It is noted by this evaluator that no statistical analysis of the data is included. The percentage inhibition for plasma ChE at the lowest dose of 0.1 mg/L is 2 and 16% for males and females, respectively. The percentage inhibition for RBC ChE is 9 and 11% for males and females, respectively.)

4) From Attachment 18: Letter from B. Dementi dated November 5, 1998

This letter comments on the October 27, 1998 draft report of the August 1998 HIARC meetings. Concerning Issue 4, Dr. Dementi does not agree with the proposed HIARC report's summary of

the panel's responses concerning question 1. He does not think Dr. Hartung's response should be interpreted as recommending against the use of an additional UF. He argues that the statement should be interpreted to mean that fine-tuning, 3x or 10x, cannot address the inadequacies. It cannot be taken to mean he opposes the factor from 3x to 10x. In addition, he says there was a follow-up response from Dr. Decker from July 21, 1998, in which he said, ***"****Based on my experience (43 years in the field of toxicology), Reference N (TES Process), and the letter from Dr. Dementi (July 9, 1998), I doubt that the 1/3 LOEL is adequate to account for the absence of a NOEL. At the present time it would seem prudent to use 1/10 LOEL. I assume, of course, that further testing will be forthcoming to determine a NOEL, at which time this safety factor should be reexamined.****"*** (The hand-written response from Dr. Decker dated July 29, 1998 and Dr. Dementi's July 9, 1998 letter requesting clarification were provided to this evaluator by Dr. Dementi on March 15, 2000.)

Dr. Dementi refutes the draft report concerning the UF. He recalls that, at the August 27, 1998 meeting, the HIARC adopted raising the UF from 3 to 10. The final December 22, 1998 HIARC report states that an additional UF of 10 was applied for the use of a LOEL and the severity of the nasal lesions for short-, intermediate- and long-term inhalation exposure. Therefore, no further discussion of the issue from this attachment will be presented.

Dr. Dementi's letter continues by stating that the HIARC decided to require another inhalation (nose-only) study in the rat. The discussion then concerns the calculation of a "derived" NOEL for nasal effects vs. ChEI effects. Since these calculations do not appear in the final HIARC document, the discussion is moot.

The letter then debates the possible NOEL/LOEL after whole body vs nose-only inhalation exposure, which this evaluator could not understand with having attended the HIARC meetings.

<u>Evaluation by the External Peer Review Panel</u>

Five questions concerning Issue 4 were presented to the external peer review panel. The <u>first question</u> asked if the use of an UF of 3 to compensate for the absence of a NOEL for ChEI and nasal and laryngeal effects is supportable. Dr. Decker said he didn't feel comfortable answering the question since the derivation of the UF of 3 was not clear to him. Dr. Dourson said ChEI (compartment not identified) does not appear to be statistically significant at the low dose so this is the NOEL for this endpoint. However, the nasal effects are biologically significant. The extent and severity of these lesions suggest the use of a 10-fold UF. Dr. Hartung said, "*This fine-tuning is unwarranted because of major species differences in exposure scenarios. In whole body exposures of rodents we deal with a direct nasal inhalation, an inhalation filtered through fur as rats assume a characteristic protective fetal position during the exposure, and a direct ingestion as rats seek to clean their fur by licking it. This is not comparable to the exposure scenario of the human applicator, the bystander, or the consumer. The unique nasal responses of rats have been discussed previously during the assessment of the inhalation toxicity of formaldehyde.*

In his consolidation report (Attachment 12 of February 28, 2000 letter), Dr. Dementi summarizes each panelist's responses individually as follows:

"Dr. Dourson: No. Advocates use of 10X rather than 3X uncertainty factor.

Dr. Hartung: No. Questions inhalation test procedure (whole body). Says fine-tuning (i.e., interpreted to mean use of 3X, or other factor) cannot accommodate gross deficiencies.

Dr. Decker: Says does not understand derivation of 3X uncertainty factor."

He then comments, "*Given the inability for Dr. Decker to respond, taken in concert with the negative responses of Drs. Dourson and Hartung, the consensus of the external reviewers is that use of a mere 3X uncertainty factor is inadequate."*

The December 22, 1998 HIARC report summarizes the panel's responses as, "*One member recommended against the use of additional UF, another, recommended a UF of 10, while the third member did not feel qualified to answer this question."*

In summary (evaluator's opinion), Dr. Dourson recommended a 10x UF. Dr. Hartung's response was unclear and did not answer the question directly. Dr. Decker's follow-up response of July 21, 1998 was not included in Dr. Dementi's or the HIARC's summary of the panel's responses. See Attachment 18 above for discussion. He said it would be prudent to use 1/10 LOEL.

The second question asked if the 2 week range-finding inhalation study, in which there was no NOEL for ChEI and nasal and laryngeal histological effects occurred at doses as low as 0.54 mg/L, should influence the HIARC decision not (emphasis added) to evoke an UF for acute risk assessment (1-7 days) on the basis of cumulative effects. Dr. Decker said that since range-finding studies seldom generate reliable NOELs, the study should not be used in deciding an UF. Dr. Dourson said comparison of the LOELs for plasma or RBC ChEI and the histopathology between the 2 week and subchronic studies at doses of 0.45 mg/L and 0.54 mg/L gives comparable results. He stated that he thinks this supports the contention that an extra UF is not needed for potential cumulative effects. Dr. Hartung said the same objections for question 1 apply to this question.

In his consolidation report (Attachment 12 of the February 28, 2000 letter), Dr. Dementi summarizes the panel's responses as follows:

"Dr. Dourson: Yes (implied). Presents the argument that comparative findings in the 2-week and 90-day studies do not support a very remarkable cumulative response, and thereby, perhaps unwittingly, dismantles the Hazard ID Committee's principal argument for not invoking the uncertainty factor in the case of short-term exposures.

Dr. Hartung: No. Same comment as in question 1

Dr. Decker: Says a rangefinding study should not be used to decide, since such studies do not provide reliable information."

In his comments section, Dr. Dementi states, "*Given the nature of responses from all three reviewers, I believe the question was not particularly clear. The Hazard ID Committee advocated a 3X uncertainty factor for the intermediate and long-term, but not for short-term(1-7 days) exposure risk assessments. The decision for not invoking the factor for the short-term exposures was predicated on the assumption that the end points in question identified in the 90-day inhalation study were cumulative in nature, and would not likely occur following the shorter term exposures. However, upon retrieving the 2-week rangefinding inhalation study, which was not available to the Hazard ID Committee at the November 6 meeting, it became clear that cholinesterase inhibition and, particularly, nasal and laryngeal hyperplasia were evident after only two weeks, and thus the argument for not applying the uncertainty factor for short-term exposures could no longer be supported. (See Refs. O and* ***CC****) Indeed, Dr. Dourson expresses the view that the end points in question may not be particularly cumulative based upon similarities of responses in the 2-week and 90-day studies. I generally agree with Dr. Decker that range-finding studies perhaps do no often provide reliable information, but in this case the range-finding study is of higher quality than most such studies, and I believe to be suitable to the extent of revealing early onset of the nasal tissue effects, and cholinesterase inhibition. So while the reviewers did not clearly address the question as to whether the uncertainty factor should be used in the case of the short term (1-7 days) exposures, the question stands, begging a response from the Hazard ID Committee."*

The December 22, 1998 HIARC report summarized the panel's responses as, "*Conclusions from two members suggests that the cholinesterase inhibition is well characterized and that an extra UF is not warranted. The third member recommended against using this study since such studies (range finding) do not provide reliable information.*"

In summary (evaluator's opinion), the ambiguity of question lead to indirect responses that could be interrupted multiple ways. Dr. Decker said the range-finding study should not influence the HIARC decision. Dr. Dourson said the study supports the contention that an extra UF is not needed for potential cumulative effects. Dr. Hartung didn't answer the question directly.

The third question concerned whether another study should be required to identify the NOEL for the endpoints in question. Dr. Decker said yes. Dr. Dourson suggested that the incidence of nasal and laryngeal effects of similar severity be plotted as a function of exposure, which would allow the use of a benchmark concentration approach. This approach could also be applied to ChE effects, however as indicated in question 1, he believes the NOEL for this endpoint has been established in the study. Dr. Hartung said, "*Not with rats on these issues.*"

In his consolidation report (Attachment 12 of February 28, 2000 letter), Dr. Dementi summarized the panel's responses as follows:

"Dr. Dourson: Yes (qualified). Suggests first using bench dose approach.

Dr. Hartung: "Not with rats on these issues." (p. 9)

Dr. Decker: Yes. "Common sense dictates that NOELs be identified." (p. 6)"

In the comments section, Dr. Dementi said, "*Comments: Dr. Dourson evidently recognizes the need to more fully characterize the responses, i.e. a deficiency exists as it currently stands. Perhaps someone expert in this area could be commissioned to perform the tasks he suggests, and lets see what it shows. Dr. Hartung questions the utility of the inhalation study. However, the Agency requires the study and it is necessary that we assess the results. Dr. Decker most clearly enunciates what should be the Agency's position, which is to identify the NOELs on this very important end point for a very important route of exposure. It should be noted that in DER # 1 an extensive discussion is presented, indicating the very remarkable metabolic capability of the nasal olfactory epithelium and includes discussion as to why malathion may be a good candidate chemical to elicit nasal effects following metabolic conversion by the nasal tissues. "*

The December 22, 1998 HIARC report summarized the panel's responses as, "*One member would like to identify a NOEL, while the other suggests first using bench mark approach. The third does not want an inhalation study with rats."*

In summary (evaluator's opinion), Dr. Decker said another study is needed. Dr. Dourson recommended an alternative approach, i.e. analyzing the available data. Dr. Hartung's response is of little value since he didn't elaborate on his position that another study shouldn't be done in rats. The question is moot since another inhalation study is required, according to the December 22, 1998 HIARC report.

The fourth question concerned whether a carcinogenicity study by the inhalation route (e.g. inhalation exposure for the first 90 days of a two year study) should be required. Dr. Decker said yes because malathion is usually sprayed so the inhalation route is most important. Dr. Dourson said, "*Perhaps one should first ask for mechanistic studies. It may be more useful to understand the mechanism of nasal injury in eventual extrapolation (by way of a linear or a MOE approach as per draft agency cancer guidelines), rather than spend the time and money on another two year bioassay*." Dr. Hartung said there are questions about whole body exposure and its validity for human health risk assessment. Nose-only studies pose so many additional stresses on the rat that interpretation of the results is confounded.

In his consolidation report (Attachment 12 of February 28, 2000 letter), Dr. Dementi summarizes the panel's responses as follows:

"Dr. Dourson: Yes (qualified). As in his response to the previous question, he says first ask for mechanistic studies to understand nasal injury. Use extrapolation via cancer guidelines.

Dr. Hartung: No answer. Still questions utility of inhalation studies.

Dr. Decker: Yes."

In his comments section, Dr. Dementi states, *"Dr. Dourson recognizes the need to address the issue, but proposes as a first alternative pursuit of mechanistic studies and extrapolation techniques. Perhaps someone expert in this area should be assigned the task and lets see what it shows, but I am not certain the most critical mechanism is identifiable with any certainty. Actual testing may be the best and perhaps only way to obtain satisfactory results. Dr. Decker is clear in his response that the study should be pursued. At other places in his response, Dr. Decker says: "The appearance of rarely-found malignant tumors in the nasal turbinates of 2 female rats should be a pointer that more animals should be tested to determine the incidence of said tumors in all dosage groups." (p. 2) We should note one of the rats in question had a carcinoma while the other had an adenoma of the olfactory epithelium. Were his suggestion to be followed, the inhalational route of exposure may be preferred, particularly if the study could be conducted in a manner acceptable to Dr. Hartung."*

The December 22, 1998 HIARC report summarizes the panel's responses as, *" One member said yes to requiring this study, another member does not want this study and the third member would like to see mode of action studies to understand nasal injury and questions the utility of the inhalation study."*

In summary (evaluator's opinion), Dr. Decker said a carcinogenicity study by the inhalation route should be done. Dr. Dourson said it would be more useful to do mechanistic studies. Dr. Hartung didn't answer the question directly.

The fifth question asked whether the study provided any support for discounting a 10x safety factor imposed under FQPA for the protection of infants and children, other than contributing to the completeness of the malathion data base. Dr. Decker said not in his opinion. Dr. Dourson said the study does not test the toxicity of malathion in rat pups or young rats and by analogy in infants and children. He refers to his answer to question 6 under Issue 3, which includes a philosophical discussion of "safety" vs "uncertainty" factors. He considers the 10x safety factor a risk management tool. Dr. Hartung answered no to the question.

In his consolidation report (Attachment 12 of the February 28, 2000 letter), Dr. Dementi summarizes the panel's responses as follows:

"*Dr. Dourson: No. Acknowledges study does not evaluate young individuals. Asserts the FQPA 10X factor to be a risk management tool.*

Dr. Hartung: No.

Dr. Decker: No."

In his comments, he states, *"The external reviewers agree the study does not provide any support for discounting use of the 10X safety factor imposed under FQPA."*

The December 22, 1998 HIARC report summarizes the panel's responses as, *" The panel agreed that the study does not provide any support for discounting use of the 10x safety factor imposed by FQPA. One member acknowledged that the study does not evaluate young individuals and asserted that the FQPA 10x factor is a risk management tool and including it in the scientific discussion of database sufficiency is not appropriate."*

In summary (evaluator's opinion), Drs. Decker and Hartung said the study doesn't provide support for discounting the 10x safety factor. Dr. Hartung said the study didn't test young animals.

December 22, 1998 HIARC Report

Concerning question 1, the HIARC concluded:

"*The HIARC concluded that a Margin of Exposure of 1000 is required for Short-, Intermediate- and Long-Term inhalation exposures. The MOE of 1000 includes the conventional 100 and an additional 10 for the use of a LOEL and the severity of the nasal lesions.*

This decision was based on the results of a two-week range finding study (MRID No. 44554301) which was not available to the Committee at the November 6, 1997 meeting. In that study, there was a dose-related increase in the lesions of the nasal cavity (hyperplasia and respiratory epithelium) which was similar to the laryngeal and nasal cavity lesions seen in the subchronic study."

Concerning question 2, the HIARC concluded:

"The HIARC concluded that based on the availability of the new data (the range finding study), a MOE of 1000 is required also for Short-term inhalation risk assessment (previously it was determined that a MOE of 100 is adequate for this exposure period)."

Concerning question 3, the HIARC concluded:

"The HIARC determined that a new inhalation study is required based on the results of the two-week range-finding study (MRID No. 44554301) and the lack of a NOAEL for cholinesterase inhibition in the 90-day study (MRID No. 43266601)."

Concerning question 4, the HIARC concluded:

"*At its meetings held on September 24, October 8 and October 15, 1997, HED's Cancer Assessment Committee (CARC) determined that in order to conduct an accurate assessment on the relevancy of nasal tumors to malathion exposure, the nasal tissues from all animals from all*

dose groups in the 2-year carcinogenicity study (MRID No. 43942901) should be evaluated/re-evaluated (Memorandum: J. Rowland, to M. Ioannou, dated 11/3/97; HED Document No. 012374). ***Therefore, the HIARC concluded that the need for a study will be determined after CARC's review and evaluation of the requested histopathological examinations."***

Concerning question 5, the HIARC concluded:

"*This study is not appropriate for FQPA assessment because: (I) the study was conducted in adult animals; (ii) there was no exposure to pregnant animals nor was there pre/post natal exposure; (iii) this study did not evaluate parameters in fetuses or pups; and (iv) is not appropriate for assessment of increased susceptibility under FQPA provisions..* ***Therefore, HIARC concluded that discussion about the FQPA Safety Factor is neither applicable nor appropriate for this study. In addition, the FQPA Safety Factor, when required, is not applied to any single toxicity study but rather for dietary and residential exposure risk assessments.***"

Evaluator's Comments on Accuracy, Clarity and Transparency of HIARC Reports

1. In the December 17, 1997 HIARC report, under C. Occupational/Residential Exposure, 5. Inhalation Exposure (Any-Time period), it states that for overall ChEI, a NOEL was not established for plasma and red blood cells. However, in the December 22, 1998 HIARC report, under Question 3 for Issue IV, it states that there was a lack of a NOAEL (as opposed to NOEL) for ChEI in the 90-day study. In responding to question 1, Dr. Dourson said that he believed a NOEL for ChEI was established in the study. The HIARC report is unclear as to the Committee's final decision about this endpoint and the basis for it.

2. In the December 22, 1998 HIARC report, under question 3 of Issue IV, the HIARC concluded that a new inhalation study is required, however it is unclear as to its duration and method of administration, i.e., whole body or nose-only.

3. In the December 22, 1998 HIARC report, the last sentence under Question 5 is unclear. The question did not ask if a 10x should be applied to the study, as indicated in this sentence.

Evaluator's Comments on HIARC Reports Reflecting Dr. Dementi's Opinions on Issue 4

1. There is evidence that the HIARC responded to Dr. Dementi's concerns about a 10-fold rather than 3-fold UF for short-term inhalation exposure and the need for an additional inhalation study to identify the NOEL, even against the recommendations of some of the external peer review panel.

2. In his February 28, 2000 letter, Dr. Dementi states that he is concerned as to how soon following malathion inhalation exposure, effects would be seen in the nasal mucosa and that the HIARC affirm the importance of determining this endpoint in the new inhalation study. This evaluator could not find this concern expressed in any of the attachments to the December 22, 1998 HIARC report. Therefore, it is not clear if this concern was presented to the HIARC during the August 1998 meetings.

3. Dr. Dementi's concern in his February 28, 2000 letter about the requirement of a carcinogenicity study via the inhalation route is under the purview of the CARC, as indicated in the 1998 HIARC report.

4. In his February 28, 2000 letter, Dr. Dementi states that he does not agree with the HIARC's summary of the panel's responses/comments. He refers to Attachment 18, page 150, in which he discusses the interpretation of Drs. Decker and Hartung's responses to question one. Dr. Decker's response (discussed under Attachment 18) was in response to a follow-up question from Dr. Dementi. Since this response is not included in the December 22, 1998 HIARC report with Attachment 1: Evaluation of External Peer Review Members or Dr. Dementi's consolidation of the panel's responses (Attachment 12), it is unclear is this information was available to the HIARC. Concerning Dr. Hartung's response, it was unclear, indirect and subject to misinterpretation by both Dr. Dementi and the HIARC.

ISSUE 5: Acute Neurotoxicity Study (Retinal Histopathology)

Dr. Dementi's Position as Summarized in February 28, 2000 Letter:

The final HIARC report rejected my recommendation for the submission of a selected few retinal slides for further histopathology assessment, as well as my recommendation that retinal slides from lower dose group animals be examined. These questions were among those submitted to the External Peer Review Panel. The Panel members were provided HED's December 7, 1997 ad hoc report along with the complete set of DERs for consideration. Their decision was that the slides bearing retinal rosette should be submitted for independent diagnosis/characterization, and that the lower dose group (s) in the study should be examined histopathological ly. Since receiving the External Peer Review results, the HIARC has offered no new reasons to rebuff the external toxicologists recommendations.

Cited in Dr. Dementi's letter: **057701ha.002:** pp. 12-13; **Att 1**; **Att 2:** p. 61, 68-72, ; **Att 4**; **Att 7**; **Att 12:** pp. 131-132.

Additional Information from Dr. Dementi's Detailed Memoranda/Letters

1) From Attachment 4: Letter from B. Dementi dated November 20, 1997

This letter concerned the ad hoc neurotoxicity subgroup meeting of November 13, 1997. Dr. Dementi maintains that the slides should be requested for the following reasons (as interpreted by this evaluator):

1) the acute neurotoxicity study guidelines call for sequential evaluations of specific tissues in lower groups when histopathological findings are noted in the high dose animals. Although only one animal was affected in the high dose, there were only five animals/sex/group examined.

2) the lesion is rare according to historical data bases

3) there is uncertainty about the anatomic features of the lesion; the term could be used to describe a variety of morphological changes

4) there are concerns about possible retinal effects of organophosphates in general and malathion in particular.

2) From Attachment 7: Letter from B. Dementi dated January 15, 1998

This letter provides additional information from Dr. Dementi. Four literature references are cited which he states indicate the terms retinal rosette, retinal fold and retinal detachment may apply to the same anatomic condition. He then presents the possible pathogenesis of the condition from a reference he acknowledges is older (from 1933). The article proposes there is a relationship

between the maintenance of normal intra-ocular pressure and the appearance of retinal rosettes.

Additional information includes a discussion of the precautionary labeling for the human drug phospholine iodide, an ophthalmic solution containing echothiophate iodide, an organophosphorothioate ChE inhibitor. Under the Adverse Reaction section, the labeling states that retinal detachment has been reported in a few cases during use of the drug in patients without a previous history of the disorder. In addition, it warns that echothiophate potentiates other ChE inhibitors, such as organophosphate and carbamate insecticides.

Dr. Dementi concludes that the additional information indicates, *"...that: 1) retinal rosettes, retinal folds and retinal detachments (even microretinal detachments) may be different terms for a common underlying effect; 2) maintenance of intra-ocular pressure may play an essential role in preserving retinal structure; 3) substantial declines in intra-ocular pressure could in principle elicit retinal detachment and/or scrolling of the retina; 4) organophosphate medicinals used to control intra-ocular pressure in the treatment of glaucoma, presumably when used with precise dosing under the care of a physician, have as an associated precaution retinal detachment; 5) malathion as evaluated in the acute neurotoxicity at single very high doses via oral gavage, in fact could have elicited a precipitous decline in intra-ocular pressure resulting in the retinal anomaly; 6) malaoxon, the active metabolite of malathion, like echothiophate is an organophosphorothioate. So whatever the mechanism of the possible association between treatment with echothiophate and retinal detachments in humans might be, that mechanism could in principle operate in the acute neurotoxicity study where very large doses were used*."

3. From Attachment 18: Letter from B. Dementi dated November 5, 1998

Attachment 18 was not cited by Dr. Dementi for Issue 5, however it is applicable to a discussion of this issue. In this letter, he responds to the HIARC's October 28, 1998 draft report of the August meetings. Concerning Issue 5, Dr. Dementi states that he finds it regrettable that the HIARC did not acknowledge or address additional comments dated January 15, 1998, which he provided to the HIARC. He further adds that by presenting only the conclusions of the ad hoc subgroup, the reader is not afforded the benefit of his ideas brought to the table at the meeting.

<u>Evaluation by the External Peer Review Panel</u>

Two questions concerning Issue 5 were asked of the external panel. The <u>first question</u> was whether retinal histopathology data should be submitted for rats in the intermediate dose group. Dr. Decker responded, "*Yes, histopathology data is generally desirable for <u>all</u> dosage groups."* Dr. Dourson thought that it was reasonable to first ask for the histopathology on three animals (identified in the December 3, 1997 ad hoc subgroup report as affected high dose male and random control male from acute neurotoxicity study and affected control rat from subchronic neurotoxicity study) before additional groups were requested. In addition, he said the effort to explore this effect might be of academic interest but has little relevance to the determination of the critical effect. (He previously said the critical effect after a single dose was ChEI.) Dr. Hartung said, "*Yes, for qualitative analysis, only. The reason for this is that either the dosing schedule or*

the cholinesterase analyses were deficient in this study. But, retinal detachments as potential end-points are sufficiently serious to merit following up on the present anecdotal finding."

In his consolidation report (Attachment 12 of February 28, 2000 letter), Dr. Dementi summarized the panel's responses as,

"*Dr. Dourson: Suggests first requesting submission of slides in question and then decide whether to evaluate lower dose groups.*

Dr. Hartung: Yes

Dr. Decker: Yes"

The December 22, 1998 HIARC report summarizes the panel's responses as, "*Two of the members said yes. The third member suggested that the decision to evaluate lower dose groups be made after re-evaluation of the slides in question".*

In summary (evaluator's opinion), Drs. Decker and Hartung said yes. Dr. Dourson suggested the decision to evaluate the lower dose groups be made after an evaluation of slides from three animals (identified in the December 3, 1997 ad hoc neurotoxicity subgroup report).

The second question asked if the histopathology slides should be submitted for independent examination by the Agency's pathologist (for anatomic features comparisons between control and treatment group lesions). [As clarification, the slides referred to are those in which retinal lesions were either observed (control animal in subchronic neurotoxicity study and high dose animal in acute neurotoxicity study) or were from control animals for comparison.] Review of the detailed responses of each toxicologist indicates all three answered yes to this question.

In his consolidation report (Attachment 12 of the February 28, 2000 letter), Dr. Dementi summarized the panel's responses as follows:

"Dr. Dourson: Yes

Dr. Hartung: Yes (evaluate the matter by either approach)

Dr. Decker: Yes"

In his comments section, Dr. Dementi said, "*All three reviewers share an opinion that additional work is indicated, the question is whether the work called for in both questions should be pursued. Dr. Decker says yes to both, while Dr. Dourson suggest that examining lower dose groups would be contingent upon the results of the independent histopathology examination proposed. Dr. Hartung advocates additional work to resolve the question. If it cannot be determined by the Agency's pathologist(s) whether the retinal finding in the high dose male group is dosing related, then it is important to acknowledge that the Guidelines require*

examination of lower dose groups."

The December 22, 1998 HIARC report gives the panel's response to this question as, *"All three members responded yes."*

In summary (evaluator's opinion), all members of the panel responded yes to this question.

December 22, 1998 HIARC Report

Concerning question number one, the HIARC concluded:

The HIARC noted that this issue of reexamination of the retinal tissue of three rats was addressed by an ad hoc subgroup of neurotoxicity experts in HED.

The ad hoc group met on November 13, 1997, and after careful evaluation of all available data, concluded that the Agency should not ask for evaluation of the retinal tissue of three rats in the acute and subchronic neurotoxicity studies. This decision was based on the following weight-of-evidence considerations (Memorandum: E. Budd to R. Loranger, dated December 3, 1997).

> *1) The lesion of concern (bilateral retinal rosette) occurred in only one male rat at the high dose in the acute neurotoxicity study.*
>
> *2) A unilateral retinal rosette was also tentatively observed in one male rat in the control group in the subchronic neurotoxicity study.*
>
> *3) Dr. Brennecke (HED's pathology consultant) and Dr. Dahlgreen (the study pathologist) both concluded the retinal rosette in the male rat at the high dose was not of toxicological significance and was not due to treatment with malathion*
>
> *4) The ad hoc group also concluded that retinal rosettes in rats are most likely the result of abnormal proliferation and differentiation of developing retinal cells during neonatal life (i.e., during the first approximately 32 days after birth) and ordinarily are not likely to develop in mature animals as a result of treatment with xenobiotics.*
>
> *5) An available reference (Ophthalmic Pathology of Animals, Saunders and Rubin, 1975), stated that "[retinal] rosettes occur spontaneously in certain strains of inbred rats and in beagle and collie dogs".*

Based on this information, the HIARC differed with the Panel's recommendations and reaffirmed the ad hoc group's decision on this issue, concluding that no additional histopathological examination is necessary at this time.

Concerning question number 2, the HIARC concluded:

The HIARC again differed with the panel and reaffirmed the decisions made by the ad hoc group based on the rationale provided above.

Evaluator's Comments on Accuracy, Clarity and Transparency of HIARC Reports Concerning Issue 5

1. Essentially the same rationale was provided for not requesting slides in both the November 17, 1997 and December 22, 1998 HIARC reports. The rationale for rejecting the panel's response to the questions in the 1998 report is simply given that the HIARC differed with the panel's recommendations based on the ad hoc group's decisions. More detail about the actual discussion of this issue at the August 1998 meetings would make the HIARC's decision clearer.

2. The document should indicate what the Agency's position is on these lesions. The 1997 HIARC report (III. FQPA Considerations, 1. Neurotoxicity Data) refers to them as a equivocal neuropathological finding. The December 22, 1998 HIARC report should note if the August 1998 HIARC discussions altered this conclusion. It is also noted by this evaluator that other lesions were reported in this study and designated as equivocal neuropathological findings. The high dose male animal also had axonal degeneration in the lumbar root. Digestion chambers in the lumbar dorsal root fibers in one male and in the sciatic and tibial nerve in another male were observed. These lesions were also designated as equivocal findings.

Evaluator's Comments on HIARC Reports Reflecting Dr. Dementi's Opinions on Issue 5

The record is not clear that the additional information in the letter dated January 15, 1998 (Attachment 7), was considered by the HIARC. This information was not available to the ad hoc subgroup which met on November 13, 1997. The December 22, 1998 HIARC report does not indicate that it was considered as part of the Committee deliberations. However, it was cited as a Minority Report and attached to the HIARC report. Also, it apparently was submitted to the external peer review panel.

ISSUE 6: Subchronic Neurotoxicity Study (Recommendation for Additional Behavioral Effects Testing)

Dr. Dementi's Position as Summarized in February 28, 2000 Letter

*The contrast between the NOEL of 1575 mg/kg/day on neurotoxicity end points (FOB; motor activity) in the Guideline Subchronic Neurotoxicity Study in the rat, and that of a LOEL of 38 mg/kg/day on a different set of neurotoxicity parameters (learning/memory; EEG; EMG) in a published work, Desi et al (1976), has been noted. My recommendation has been that this published work be considered of sufficient merit and concern, because of the low doses involved, to trigger a study of malathion on behavioral, learning/memory or cognitive end points not evaluated in the existing Guideline study. I have noted that cholinesterase inhibition reported in Desi et al is consistent with that in the Guideline study, which serves to enhance the credibility of both studies. In support of Desi et al, I have also cited Kurtz (1977), in which malathion was shown to elicit avoidance behavior in rats following single doses as low as 50 mg/kg (but not 25 mg/kg) and above as administered intraperitoneally. HIARC, on the other hand, discounted these published works as being of sufficient merit even to elicit further testing. According to my interpretation, two members of the External Peer Review Panel support a requirement for additional neurotoxicity behavioral effects testing, though one of these Panelists, Dr. Hartung, in reference to Desi et al, questions the reliability of "Russian neurophysiology". I should note in response that the article in question appeared in a recognized, peer reviewed, Western journal. Dr. Decker heartily supported the testing. The third Panelist, Dr. Dourson, says: "I do not believe that it does. The LOEL of 38 mg/kg-day for both cholinesterase depression and possible learning effects is not inconsistent with the cholinesterase NOEL of 4 mg/kg-day from the 2 year rat bioassay." (**Att 1**, p. 35). Dr. Dourson seems to be saying the finding is not surprising or unexpected, results in the Guideline testing not withstanding.*

A journal publication [(Ehrich et al (1993)], not identified in HIARC's 1997 report: "Information from the Open Literature" (p. 63), nor subsequently by the FQPA Safety Factor Committee, reported that malathion at all single dose levels administered, the lowest being 600 mg/kg/day, yielded positive responses on EPA's FOB parameters before or by day 21 post dosing. This study in conjunction with other published works should be reviewed by HIARC and the FQPA Safety Factor Committee in its consideration of the reliability of the data base, and more specifically with respect to the recommendation for the Developmental Neurotoxicity Study, or other cognitive effects testing for malathion.

Cited in Dr. Dementi's letter**: 057701ha.002:** pp. 13-14; **Att** 1; **Att 2:** pp. 61, 63-65, 71-72; **Att 4:** pp. 104-105; **Att 6:** pp. 108-109; **Att 12:** pp. 132-134; **Ehrich**.

Additional Information from Dr. Dementi's Detailed Memoranda/Letters

1. From Attachment 4: Letter from B. Dementi dated November 20, 1997

This letter responds to the ad hoc neurotoxicity subgroup meeting on November 13, 1997. Under Testing for Effects on Learning/Memory, Dr. Dementi discusses the Desi et al (1976) study in which doses of 38 or 75 mg/kg/day in a subchronic study elicited effects on learning/memory and EEG and EMG measurements. He contrasts these findings to the guideline subchronic neurotoxicity study where no neurotoxic effects were observed at doses of up to 1575 mg/kg/day. Dr. Dementi recommends that a guideline study of learning/memory be required for malathion. He says the ad hoc neurotoxicity subgroup rejected the recommendation on the grounds that the Desi et al study was not reliable. Dr. Dementi then discusses the findings of the study. He maintains that the ChE findings were consistent with the subchronic neurotoxicity study, which enhances the credibility of the published study. In addition, adverse effects of malathion on kidney tissue *in vitro* is somewhat consistent with or supported by chronic nephropathy as the cause for mortality in the 1996 combined chronic toxicity/carcinogenicity study. (It is unclear if the *in vitro* findings were from the Desi et al study.)

Dr. Dementi said the ad hoc neurotoxicity subgroup was mute in acknowledging this supporting evidence. The group criticized the Desi et al study on the grounds that the effects on learning/memory in the maze studies were small, not dose-related between 38 and 75 mg/kg/day, the statistics were ill-defined and it would be surprising for malathion to exert this effect at such a low level. Dr. Dementi says he explained that the findings were not small in terms of differences in errors made in dosed groups vs. controls. He says he offered his opinion that 38 and 75 mg/kg/day, when compared to the shallow dose response curve for malathion, are not very different. Brain ChEI was 20% in the two groups at 21 days, the time at which learning/memory was affected. He refers to the study analyses in an earlier study by Desi et al, which if comparable to the 1976 study, would make the difference between control and treated groups statistically significant. Dr. Dementi says that, despite the learning/memory findings, plus the EEG and EMG data affirming a neurological effect, and in view of the fact that the subchronic neurotoxicity study does not measure many of these effects, the ad hoc neurotoxicity subgroup categorically rejected the Desi et al study. Dr. Dementi concludes that, despite its deficiencies, the study is of sufficient quality and its conclusions mandate verification through proper guideline testing procedures.

2. From Attachment 6: Letter from B. Dementi dated December 17, 1997

This letter comments on the draft report of the November 6, 1997 HIARC meeting. Under B. Chronic Dietary [Reference Dose (RfD)], Dr. Dementi refers to the statement in the draft report that the NOEL for the 2-year study is supported by the 90-day study. He says that, if this is in reference to the subchronic neurotoxicity study, it is true the NOEL is 50 ppm over a 90-day period. However, that study had only 5 rats/sex/group at each time period and *"...had no other dose groups between 50 and 5000 ppm, that would demonstrate the ability of the study to detect cholinesterase inhibition within that large range."* He further states that plasma ChEI is so imprecise in that study that it is questionable whether 5000 or 50 ppm is the NOEL in either sex,

which underscores the need for a study on a large number of animals to obtain a definitive NOEL for ChEI.

Under VII Data Gaps, he includes the submission of a *"guideline behavioral test yet to be specified."*

Evaluation by the External Peer Review Panel Members

The panel was asked two questions concerning Issue 6. The first question asked if a comparison of the findings in the guideline subchronic neurotoxicity study and the Desi et al (1976) study provide an adequate reason to require a developmental neurotoxicity study that measures parameters not covered in the subchronic neurotoxicity study. Dr. Decker does not answer the question under the responses but in his comments on the DER (#10) for the subchronic neurotoxicity study. There, he says he recommends a literature search be used to construct additional neurotoxicity testing for effects on learning, behavior and EEG and EMG evaluations. Dr. Dourson says he doesn't believe it does. He compares the LOEL of 38 mg/kg/day for ChEI and possible learning effects to the ChEI NOEL of 4 mg/kg/day from the 2 year rat bioassay. He says if the LOEL was divided by a 10-fold UF, which is the standard EPA practice with LOELs of this severity, similar values would be obtained (3.8 vs. 4 mg/kg/day). The use of a 3-fold UF would make the ChEI more significant. Dr. Hartung said neurotoxicity studies are fairly easy to conduct but present difficulties in selection of proper controls and interpretation of results. He states that Russian scientists (assumed that he refers to Desi) have relied on neurophysiological measurements that have not been accepted in western nations due to difficulties in interpreting the data. He concludes that the spread between the simple behavioral response and ChEI argues against the need for further study. He states that he is not familiar with the range of studies asked for under the developmental neurotoxicity guidelines. He says, "*If this is an unrestricted list of test methods, then it should be subjected to an assessment of interpretability and utility prior to requiring its execution."*

In his consolidation report (Attachment 12 of February 28, 2000 letter), Dr. Dementi summarizes the responses as follows:

Dr. Dourson: No. His reason resides in an opinion that if the study were performed, it would not likely yield a result that would infringe the RfD.

Dr. Hartung: Yes (implied), but questions the acceptability of Russian neurophysiology (EEG, EMG) assessments.

Dr. Decker: Yes

Dr. Dementi's comments section on this question is very extensive. It supports, refutes or expands upon each panelist's responses. To avoid misinterpretation by this evaluator, the comments section is attached verbatim as Attachment 5.

The December 22, 1998 HIARC report summarized the panel's responses to question 1 as:

"One panel member said yes. One member questioned the acceptability of the published study. The other member did not believe that the published study provided reason to require additional studies."

In summary (evaluator's opinion), Dr. Dourson said no. Dr. Hartung commented on the interpretation of neurotoxicity studies and did not answer directly. Dr. Decker is the only panelist who directly recommended another study. However, the issue is moot since a developmental neurotoxicity study was required for malathion under a Data Call-In (DCI) dated September 10, 1999.

The second question asked if the neurotoxicity findings in the published study (assumed to be Desi et al 1976 study), are considered inadequate to trigger additional guideline testing, what criteria from published work might serve in this capacity. Dr. Decker suggested that a neurotoxicologist be contacted for advice on what criteria from published work should be used. Dr. Dourson said EPA must make the judgment on this issue. He continued that one must judge whether or not the expected NOEL for learning effects will be lower than the NOEL of 4 mg/kg/day for ChEI divided by the UF of 3 for data deficiencies. Therefore, if the learning NOEL should be expected to be below approximately 1 mg/kg/day before requiring such a test. Dr. Hartung repeated his comments on the difficulty in interpreting Russian neurotoxicity data.

In his consolidation report (Attachment 12 of February 28, 2000 letter), Dr. Dementi summarizes the panel's responses as follows:

"Dr. Dourson: Defers to EPA's experts.

Dr. Hartung: No answer.

*Dr. Decker: Suggests having a neurotoxicologist provide criteria***."**

In his comments section, he adds, "*The consensus opinion is to defer the question to neurotoxicologists. These also must be external peer reviewers*."

The December 22, 1998 HIARC report summarized the panel's responses to question 2 as:

"*One panel member said yes. One member questioned the acceptability of the published study. The other member did not believe that the published study provided reason to require additional studies."*

In summary (evaluator's opinion), the question was confusing and inappropriate for an external peer review panel, as indicated by their responses where two of three referred the question to someone else and the third did not answer. However, the issue is moot since the developmental neurotoxicity study is required.

December 22, 1998 HIARC Report

Concerning question one, the HIARC concluded:

"The ad hoc group, after careful evaluation of all available data, concluded that the Agency should not ask for additional neurotoxicity studies on malathion at this time. It was recognized, however, that such studies might possibly be requested at some time in the future if there is sufficient justification for doing so. The group also suggested that additional literature searches should be conducted on learning/behavior effects of organophosphates in general, and available information on malathion particularly (Memorandum: E. Budd to R. Loranger, dated December 3, 1997).

The HIARC reaffirmed the ad hoc group's decision on this issue and concluded that no additional studies are required at this time. *The HIARC also noted that lack of studies that evaluate learning and/or memory or behavioral effects under the Subdivision F Guideline requirement is a generic issue applicable to all organophosphates, and not particular to malathion. The HIARC recommended that the issue of requiring such a study should be evaluated in conjunction with discussion on the data requirements for FQPA."*

Concerning question two, the HIARC concluded:

"As discussed above, the HIARC noted that this is a generic issue that needs further discussion by OPP."

Evaluator's Comments on Accuracy, Clarity and Transparency of HIARC Reports Concerning Issue 6

The following comments are provided for completeness. This issue is essentially moot since a developmental neurotoxicity study is required for malathion under a Data Call-In (DCI) dated September 10, 1999. A requirement for a comparative evaluation of ChEI (or other biomarkers) and behavior in adults and young organisms was added to the modified developmental neurotoxicity study. In a December 20, 1999 letter from Jellinek, Schwartz and Connolly, the registrant agreed to conduct this study, with a protocol to be submitted in four months. (Copies of the DCI and registrant response were provided to this evaluator by Susan Makris on March 21, 2000.) However, if the NOEL in this study is higher than the LOEL in the Desi study, this issue may come before the HIARC again. The Desi et al (1976) study should be reviewed and evaluated by the HIARC for its usefulness in risk assessment.

1. In both the December 17, 1997 and December 22, 1998 HIARC reports, it states that an additional literature search should be conducted on the learning/behavior effects of organophosphates in general and malathion, in particular. It is unclear why this wasn't completed in the interim between the two HIARC meetings.

3. The December 3, 1997 report for the ad hoc neurotoxicity subgroup states that a memorandum from R. C. MacPhail commenting on the Desi et al study was considered as part of the deliberations on this issue, however no substance is provided. Details on Dr. MacPhail's comments would make the discussion section clearer.

Evaluator's Comments on HIARC Reports Reflecting Dr. Dementi's Opinions on Issue 6

Concerning Issue 6, there is evidence in the HIARC report that Dr. Dementi's opinions have been considered in the following:

1. In his February 28, 2000 letter, Dr. Dementi expresses his concern that published works, including the Desi et al and Kurtz (1977) studies (Kurtz study is assumed to be the one discussed under Issue 2) were discounted by the HIARC as being of sufficient merit to elicit further testing. He then discusses his interpretation of the external peer review panel's responses/comments on whether a study should be required. As stated repeatedly, the issue is moot since a developmental neurotoxicity study is required under the DCI.

4. In his February 28, 2000 letter, Dr. Dementi refers to as study by Ehrich et al (1993) not identified in the December 17, 1997 HIARC report or the FQPA Safety Factor Committee report. In this study, a single dose of malathion of 600 mg/kg/day (lowest dose tested) demonstrated effects on the Functional Observation Battery parameters before or by day 21 post dosing. This evaluator cannot find reference to this study in any of Dr. Dementi's correspondence to the HIARC so it is unclear if the Committee was aware of this study at any of the meetings. He did not mention the study in his November 20, 1997 or December 17, 1997 comments on the draft HIARC report for the November 6, 1997 meeting.

ISSUE 7: Cholinesterase Inhibition - Enhanced Sensitivity of Females

Dr. Dementi's Position as Summarized in February 28, 2000 Letter

*Information illustrating the gender specific disparity was presented to HIARC at the November 6, 1997 meeting, where a decision was rendered for the matter to be considered by an ad hoc group. The report of that ad hoc group says: "Regarding the possibly greater sensitivity of females (as compared to males) to the cholinesterase inhibiting effects of malathion, the results of cholinesterase determinations in numerous studies (emphasis added) on malathion were discussed and it was agreed that females do indeed appear to be more sensitive than males." (**Att 2**) Those "numerous" studies that were before the committee are part of the overall background materials that were available to the HIARC and the External Peer Review Panel, though are not included in this package; one exception being **Att 10**. Nevertheless, the ad hoc committee did conclude females to be more sensitive, but felt the difference was too small to merit imposition of an additional modifying factor. I disagreed with that decision concerning the magnitude of the effect as being too small to merit an uncertainty factor. Later, I concluded that a consensus exists among the External Peer Review Panel that females are more sensitive. Furthermore, there was a consensus (unanimous if the human study on male prison volunteers is retained for the RfD) among the Panelists that additional testing be performed in animal models to further quantitate the gender specific disparity. (**Att 12**) Yet, in spite of these considerations, it was my observation at the final HIARC meetings of August 1998, that the "Expert" chosen to address this issue merely proclaimed there was no gender specific disparity, while the final HIARC report: 1) proclaims things not consistent with my recollections; 2) resorts essentially to the language previously employed by the ad hoc committee; and 3) says that additional testing is not necessary. The External Peer Review has again been discounted by HIARC. The bottom line is summarized as follows: I am convinced females are sufficiently more sensitive to merit an additional modifying factor for the human (male only) study derived RfD, should that be retained. Furthermore there is both reason and precedent to employ a modifying factor when cholinesterase data in but one gender serves as the basis for an end point as important as the chronic RfD, e.g. carbofuran. (**Att 5**) Additional testing in animal models should be pursued to quantitate the magnitude of the gender specific disparity, while in the interim employing an additional 10-fold modifying factor since no data exists for women, or girls in particular.*

Cited in Dr. Dementi's letter*:* **057701ha.002:** pp. 14-15; **Att 2:** 69-70; **Att 4**; **Att 5**; **Att 10**; Att **12:** p. 134-135; **Att 18:** pp. 153, 156.

Additional Information from Dr. Dementi's Memoranda/Letters

1. From Attachment 4: Letter from B. Dementi dated November 20, 1997

This letter is in response to the November 13, 1997 ad hoc neurotoxicity subgroup meeting. Under Relative Sensitivity of Females Versus Males to Cholinesterase Inhibition by Malathion, Dr. Dementi states that, although the magnitude of differences between sexes is variable across studies, there is more than adequate evidence to establish a greater sensitivity for females. The

subgroup did agree that sex-related differences are manifest but did not concur that a "correction factor" should be applied to male data used to establish the RfD. Dr. Dementi states that the RfD (chronic) of 0.02 mg/kg/day, which protects the entire population, was obtained from a study in men only. Without data in women and youths, it is his opinion that a safety factor larger than 10 should be used, particularly in the face of evidence that females are more sensitive in laboratory animal studies. In addition, studies of OPs in general suggest young individuals are more sensitive. Using the 1998 malathion registration standard, he then illustrates that children consume a much higher percentage of the PADI. He states that a "correction factor" either could be calculated from the data base or additional studies in animals. He concludes, "*Additional study in animals may be necessary to properly identify the correction factor. Realizing that a sex-related differential sensitivity exists, unacceptable in my opinion is the Committee's out of hand rejection of the argument that a meaningful ratio exists without first obtaining some numerical estimates of that ratio of sensitivity from the data currently in hand. Indeed, I had anticipated that an outcome of the meeting would be a Committee recommendation that such estimates be computed for subsequent consideration."*

2. From Attachment 5: Letter from B. Dementi dated November 25, 1997

This letter offers further comments on the November 6, 1997 HIARC and November 13, 1997 ad hoc neurotoxicity subgroup meetings. Dr. Dementi reiterates his view that for studies wherein ChEI was obtained for one sex as in the human study where only male volunteers were tested, a greater than 10 uncertainty factor should be applied. He refers to the 1997 RED toxicology chapter for carbofuran in which the Agency applied an uncertainty factor of 100 to the NOEL for ChEI in male volunteers. He quotes from that Reregistration Eligibility Decision (RED) toxicology chapter: "An uncertainty factor (UF) of 10 was applied to account for intra-species variability. An additional UF of 10 was applied to account for study deficiencies (use of limited number of subjects, few subjects/dose and **use of males only** (emphasis added)". He notes that the human study with malathion has its inadequacies, including a limited number of subjects, unknown purity of the test material, interpretation of low and mid dose effects confounded by administration of EPN. He states that at an earlier time point, an uncertainty factor of 100 was applied to the human study to result in an RfD of 0.002 mg/kg/day. (The source of this information is not cited.) He recommends that the HIARC seek the historical record on the setting of the RfD for malathion and "*...make your own independent assessment of its reasonableness."* He states that, if an uncertainty factor of 100 is appropriate for carbofuran for the reasons given, an explanation should be offered on the use of 10 for malathion.

3. Attachment 10: Letter from B. Dementi dated March 16, 1998

This letter is identified as an addendum to his December 17, 1997 comments on the HIARC report for the November 6, 1997 meeting. The comments concern a 2 week range-finding inhalation study cited in his March 10, 1998 memorandum. Dr. Dementi asserts that, in this study, at doses of 0, 0.56, 1.58 and 4.23 mg/L, a NOEL was not identified for RBC ChEI in either sex or for plasma or brain ChEI in females. He presents the plasma, RBC and brain ChE data for males and females from this study and the 90-day inhalation study in a table. He concludes that

the two studies together indicate that RBC ChE is equally responsive in both sexes but that females are more remarkably affected in terms of plasma and brain ChEI. He states that the range-finding data strengthen the conclusion in the subchronic study that there is no NOEL for plasma ChEI in females and possibly for RBC ChEI in both sexes. (It is noted by this evaluator that no statistical analysis of the data is included. The percentage inhibition for plasma ChE at the lowest dose of 0.1 mg/L is 2 and 16% for males and females, respectively. The percentage inhibition for RBC ChE is 9 and 11% for males and females, respectively.)

4. From Attachment 18: Letter from B. Dementi dated November 5, 1998

This letter refers to the November 5, 1998 draft HIARC report. Concerning Issue 7, the following comments are provided by Dr. Dementi:

P13, paragraph 7: The HIARC report states that, "*...the entire data base should be examined to see if any pecularities exist that could serve as a basis for claims of sex-linked sensitivity*." Dr. Dementi states that he agrees with this conclusion and trusts there will be follow-up.

P14, paragraph 1: Commenting on the statement that there is no consistent difference in sensitivity of males vs. females, Dr. Dementi says the HIARC failed to cite the November 13, 1997 ad hoc neurotoxicity committee report, which concluded females are more sensitive.

P 14, paragraph 6: Dr. Dementi says the summary of the panel's response concerning question 3 (whether additional testing should be done to quantitate the gender disparity) should state that one member who answered no qualified his response to as long as the rat study served as the basis for the RfD.

Evaluation by the External Peer Review Members

The external peer review panel was asked three questions concerning Issue 7. The first question was whether the malathion data base supports a conclusion that females are the more sensitive gender with respect to ChEI. Dr. Decker said that there seems to be enough in the malathion data base to support this conclusion. He then refers to his answer to question 3 in which he said more data are needed to define the gender-specific disparity. Dr. Dourson said, "*The data suggest that this might be the case in the area of dose where cholinesterase inhibition occurs. What is perhaps more important, however, is the value of the NOEL for cholinesterase inhibition in both sexes. As I have stated elsewhere in these comments, I believe that the NOEL for cholinesterase inhibition is the same for both sexes in the 2 year rat bioassay. Thus, no additional uncertainty factor is needed for this possible increased sensitivity because the RfD is based on the NOEL."* Dr. Hartung said the data were not assembled in a way that would allow ready assessment of this question. He said the data in specific DERs (numbers 1, 3, 4, 9, 10 and 12) should be plotted and tested for differences. He said the data, as summarized in Refs. W, Y and Z (not included in materials available to this evaluator) indicate that females are equally sensitive in some cases and more sensitive in others.

In his consolidation report (Attachment 12 of the February 28, 2000 letter), Dr. Dementi presents a summary of each toxicologist's response as follows:

> *Dr. Dourson: Says maybe yes, but not so in the 2-year study now recommended by the Hazard ID Committee as the basis for the RfD.*
>
> *Dr. Hartung: Says data are not presented in proper manner for his assessment.*
>
> *Dr. Decker: Yes, more data needed to characterize the gender specific disparity*

In his comments, Dr. Dementi proposes the possibility of a follow-up with Dr. Hartung in the event resolution is not achieved without his comments. He then concludes that a consensus is that females are more sensitive based on responses to this question and other questions on this issue.

The December 22, 1998 HIARC report summarizes the panel's responses as:

One member says may be yes, but not in the two-year study used for establishing the RfD. The second member stated that the data are not presented in a proper manner for this assessment. The third member responded that yes, more data is needed to characterize the gender specific disparity.

In summary (evaluator's opinion), Dr. Decker said yes, the data support the conclusion that females are more sensitive. Dr. Dourson said this might be the case where ChEI is concerned. Dr. Hartung said the data are not assembled in a way to allow ready assessment.

The second question concerned what approach might be taken to estimate from the data currently available, a "correction factor" to be applied to the NOEL from the human study to afford equivalent protection for women. Dr. Decker said he could not answer the question since it was not his field of expertise. Dr. Dourson said, *"Based on the possible difference in the extent of cholinesterase inhibition between male and female rats at the LOELs in the 2 year bioassay, the NOEL/LOEL range in female humans could potentially be lower. Alternatively, the male human study is by gavage; thus, the dietary NOEL/LOEL range in human males could potentially be higher. These uncertainties might be quantifiable based on an analysis of other data for this chemical, or perhaps related chemicals, but they do operate to cancel each other out. The end result would be that the expected human female dietary NOEL might be of the same relative value as the existing human male gavage NOEL."* Dr. Hartung said the existing data should be explored to determine if gender-mediated sensitivities are universal across species. If so, he asked what is the magnitude (ratio) of the difference. He then added that if a ratio can be discerned and defended, it can be applied to data applicable to the human male.

In his consolidation report (Attachment 12 of the February 28, 2000 letter), Dr. Dementi summarizes each panel members as follows:

> *Dr. Dourson: Equivocal. Does not support the effort if the human study is not used.*

Dr. Hartung: Supports evaluating the data base for the male/female ratio of sensitivity.

Dr. Decker: Says not his area of expertise.

In his comments section, Dr. Dementi says, "*The reviewers appear to recognize the importance of the task, but are not certain how to approach it.*"

The December 22, 1998 HIARC report concluded, "*The members were split on this issue and did not offer any concrete approach to this."*

In summary (evaluator's opinion), Dr. Decker didn't answer the question. Dr. Dourson compared the NOEL/LOEL in the rat and human studies. Dr. Hartung said the data should be explored, a ratio calculated and applied to the male.

Question 3 asked if additional testing in animal models should be required to further quantitate the gender specific disparity. Dr. Decker said more data are need to rigorously define the gender-specific disparity. Dr. Dourson said no because the NOELs for ChEI in male and female rats are the same in the critical study. Dr. Hartung said,
"*1.- Do a thorough analysis of existing data first, as suggested for question 2.*
2.- Add another species, if necessary.
3.- If possible, expand the Moeller study to include females."

In his consolidation report (Attachment 12 of the February 28, 200 letter), Dr. Dementi summarized the panel's responses as follows:

"Dr. Dourson: No, to the extent the human study is not used.

Dr. Hartung: Yes

Dr. Decker: Yes"

In his comments section, Dr. Dementi said, "*A consensus exists to pursue the task. If the human study is retained as the basis for the RfD, it appears the consensus would be elevated to one of unanimity."*

The December 22, 1998 HIARC report summarized the panel's responses as, *"One member said no. Another suggested the study be extended to include females. The third member said yes, more data are needed to define gender disparity."*

In summary (evaluator's opinion), Dr. Decker said more data are needed to quantitate the gender disparity. Dr. Dourson said another study wasn't needed. Dr. Hartung said to analyze the data first, add another species and expand the human testing to include females. (His response is confusing to this evaluator.)

December 22, 1998 HIARC Report

Concerning question one, the HIARC concluded:

This issue (the possibility of greater sensitivity in one sex) has surfaced several times in the past with respect to setting RfD for other chemicals and, as a general policy, it has previously been decided that an additional uncertainty factor would not ordinarily be applied to the RfD based on possible sex-related differences

In considering sex related sensitivity to malathion, the entire data base should be examined to see if any peculiarities exist that could serve as a basis for claims of sex-linked sensitivity. If peculiarities are present, they should be further examined to determine whether they are consistent in their occurrence; affecting the same endpoint, and affecting females with the same degree of sensitivity across species lines.

The toxicology profile suggests that overall sensitivity to malathion is similar for both sexes and that there is no reason to believe that females are consistently more sensitive than males. In certain studies (e.g., subchronic neurotoxicity study in rats, the subchronic inhalation toxicity study in rats and the 21-day dermal toxicity study), females do indeed appear to be more sensitive than males as there are indications that the difference in cholinesterase inhibition is at least an order of magnitude when males were compared to females However, there is no clear picture on the relative degree of increased sensitivity of females compared to males when observed. When studies in which females appeared to be more sensitive are further examined to see what compartment of cholinesterase is affected, again there is no consistency. In some cases, the red blood cell and plasma activity appears to be indicators of sensitivity and in other cases, the brain cholinesterase activity appear to be more sensitive. Again, this finding is in studies in which females were designated as having lower NOAELs when cholinesterase was the endpoint of concern. In many (but not all) studies, the sex-related difference did not result in different cholinesterase NOELs for males and females, but rather in different degree of cholinesterase inhibition for males and females at a given dose level. The HIARC noted that NOELs, rather than degree of effect at a given dose level, are used to derive the RfD.

Concerning question two, the HIARC concluded:

The HIARC concluded that even if the human study (where no females were used) had been chosen as the basis for the RfD, it would not be appropriate to apply additional uncertainty factor to account for the increased sensitivity of females as compared to males. The rationale for this decision was that (I) when sex-related difference in sensitivity was observed, the difference appears to be small and (ii) the NOELs, rather than degree of effect are used to derive the RfD. However, the RfD is based on the chronic rat study, an additional factor based on sex would be of no relevance since the NOEL for plasma cholinesterase inhibition in that study was 50 ppm for both sexes (equivalent to 4 mg/kg/day in males and 5 mg/kg/day in females). (Note: one panel member also pointed out that the "NOELs for cholinesterase inhibition in both male and female rats are the same in the critical study").

Concerning question three, the HIARC concluded:

It was the consensus of the Committee that additional testing is not necessary because: (I) the human study (with one sex) was not used for establishing the RfD; (ii) the NOELs for cholinesterase inhibition in both males and female rats are the same in the critical (animal) study used to derive the RfD (as duly noted by one of the Panel member); (iii) as discussed above, the "apparent" sex difference in sensitivity is not consistent across studies/species (some studies showed fairly large differences); and (iv) NOELs, rather than degree of effect at a given dose level, are used to derive the RfD.

Evaluator's Comments on Accuracy, Clarity and Transparency of HIARC Reports Concerning Issue 7

1. In the December 22, 1998 HIARC report, the second paragraph under HIARC's Conclusions to Question 1 is unclear in that it says the entire data base should (emphasis added) be examined to see if any peculiarities exist that could serve as a basis for claims of sex-linked sensitivity. The implication is that this examination should be done in the future. However, the next paragraph states that the toxicology profile suggests that the overall sensitivity to malathion is similar for both sexes and that there is no reason to believe that females are consistently more sensitive than males. The implication is that the entire data base was examined as suggested in the second paragraph.

2. In the December 22, 1998 HIARC report, the second paragraph under HIARC's Conclusions to Question 1, the terms NOAEL and NOEL are both used when discussing ChE findings. If the intention was to differentiate a No Observed Adverse Effect Level from a No Observed Effect Level, further explanation would make the document clearer.

3. It is unclear if any of the HIARC's conclusions about the questions for Issue 7 are based on the ad hoc neurotoxicity subgroup's conclusions.

Evaluator's Comments on HIARC Reports Reflecting Dr. Dementi's Opinions on Issue 7

1. In his February 28, 2000 letter, Dr. Dementi states he concluded that a consensus existed among the external peer review panel that females are more sensitive. In response to question one concerning whether females are more sensitive to ChEI, two of the three panel members did respond yes. The third said the data were not assembled in a way that would allow an assessment. It cannot be concluded that this is a consensus since the panel was not a collective opinion but three individual opinions.

2. Dr. Dementi's recommendation that an additional modifying factor should be used for the human study if it is used for the chronic RfD is moot as discussed under Issue 3. Since the rat study, in which there were comparable NOELs for ChEI in males and females, was used for the chronic RfD, there is no basis for additional testing. This is explained under question 3 in the December 22, 1998 HIARC report.

3. Dr. Dementi's February 28, 2000 letter states that the final HIARC report proclaims things not to his recollection from the August 1998 HIARC meetings. In his Attachment 18, he comments on the draft HIARC report from meetings. This evaluator could not find any statements about the report being a misrepresentation concerning Issue 7.

4. Dr. Dementi's February 28, 2000 letter refers to a precedent for employing a modifying factor when ChE data in one gender serves as an endpoint and cites the RED toxicology chapter for carbofuran. According to his Attachment 5, the carbofuran RfD was based on a human study. Therefore, under current EPA policy concerning human testing, this argument no longer applies.

ATTACHMENT 1: Citations from Dr. Brian Dementi's February 28, 2000 Letter

057701ha.002	Malathion: - RE-EVALUATION A Report of the Hazard Identification Assessment Review Committee dated December 22, 1998
Attachment 1	Evaluations by the External Peer Review Members
Attachment 2	Report of the Hazard Identification Assessment Review Committee (12/17/97)
Attachment 3	Letter from Brian Dementi - November 10, 1997
Attachment 4	Letter from Brian Dementi - November 20, 1997
Attachment 5	Letter from Brian Dementi - November 25, 1997
Attachment 6	Letter from Brian Dementi - December 17, 1997
Attachment 7	Letter from Brian Dementi - January 15, 1998
Attachment 8	Letter from Brian Dementi - February 10, 1998
Attachment 9	Letter from Brian Dementi - March 10, 1998
Attachment 10	Letter from Brian Dementi - March 16, 1998
Attachment 11	Letter from Brian Dementi - March 20, 1998
Attachment 12	Letter from Brian Dementi - July 27, 1998
Attachment 13	Letter from Brian Dementi - July 29, 1998
Attachment 14	Letter from Brian Dementi - August 3, 1998
Attachment 15	Letter from Brian Dementi - August 10, 1998
Attachment 16	Letter from Brian Dementi - August 17, 1998
Attachment 17	Letter from Brian Dementi - September 24, 1998
Attachment 18	Letter from Brian Dementi - November 5, 1998
Swetz99:	January 29, 1999 memo to Clark Swentzel
Ehrich:	January 18, 2000 memo to Paula Deschamp conveying Ehrich, et al (1993), not

available electronically. The publication is available under MRID 45045001.

ATTACHMENT 2: Questions Submitted to External Peer Review Panel[13]

I. Hazard Identification/Acute Oral (One-Day)

Question 1): *Do the rabbit developmental toxicity and developmental range-finding toxicity studies support a conclusion that a single oral dose of malathion as high as 50 mg/kg would be without toxicological consequence in either the maternal or the developing organism?*

Question 2): *Do data on maternal body weights and body weight gain now available in App. III of the rabbit development toxicity study, alter the assigned LOEL/NOEL for the study and does it influence the interpretation as to whether a single dose of malathion of 50 mg/kg would be without toxic effect?*

Question 3): *As presented in a published work in the open literature, a single intraperitoneal dose as low as 50 mg/kg/day in the rat reportedly elicited a clear effect on avoidance performance while cholinesterase inhibition (erythrocyte) was observed at 100 mg/kg. Plasma and brain cholinesterase were also inhibited at 150 mg/kg. Cholinesterase inhibition and decrements in behavior were all very significant though transient effects: a) What level of confidence should be accorded this study?; b) What is the implication of the route of administration to the question of whether a single oral dose of 50 mg/kg serve as an endpoint for acute dietary (one-day) risk assessment?; c) Is the data available in the developmental toxicity studies sufficiently reliable to discount the 10x safety factor required under FQPA?.*

II. Determination of Susceptibility, Reproductive Toxicity

Question 1)*: Can the evidence indicating greater sensitivity of offspring versus parental animals in the two-generation reproduction study in the Sprague-Dawley rats be dismissed as ".....not a true indication of increased sensitivity of offspring....." for the reasons stated in the Hazard ID Committee report?.*

Question 2): *In the absence of assessments of cholinesterase inhibition and behavioral effects testing in adult and young animals in reproduction studies, can the data obtained in the FIFRA guideline study be considered adequate to address the question of whether young or mature animals are more sensitive to malathion?.*

Question 3): *Does this two-generation reproduction study provide the reliable evidence of no increased sensitivity in pups when compared to adults, as required under FQPA, to discount the 10x safety factor imposed by FQPA as additional protection for infants and children?*

III. Hazard Identification/Chronic Dietary (RfD)

Question 1): *Given the evidence of a post 3 month recovery of erythrocyte cholinesterase inhibition in females in the combined chronic toxicity/carcinogenicity study in the rat, can 50 ppm be concluded to*

[13] The questions are taken from the December 22, 1998 HIARC report.

have been a NOEL for the first three months of testing?

Question 2): *Alternatively, do these findings suggest flawed cholinesterase methodology, and if so, what corrective measure could be pursued?*

Question 3): *Should 4 mg/kg/day, the NOEL for plasma cholinesterase inhibition in males, be supported as a replacement for human data previously relied upon in establishing the RfD, or should additional testing be required in the rat to identify a NOEL for cholinesterase inhibition, particularly in females?*

Question 4): *Given that an explanation exists for greater sensitivity of humans than rats with respect to cholinesterase inhibition from malathion exposure (i.e., the lack of carboxylesterase in human plasma) should a 10x safety factor applied to the rat data to allow for "uncertainties" in inter-species variability be considered adequate if the rat data is to be used in deriving the RfD?*
Question 5): *Further, given the RfD based on human data (0.023 mg/kg/day) is lower than that derived from the rat data (0.040 mg/kg/day) and that an explanation exists for a greater sensitivity for humans, should the RfD based on human data be retained?*

Question 6): *Other than contributing to the completeness of the malathion data base, does this study provide any support for discounting a 10x safety factor imposed under FQPA for the protection of infants and children?*

IV. Subchronic Inhalation Study

Question 1): *Is the use of a UF (uncertainty factor) of 3 to compensate for the absence of a NOEL for cholinesterase inhibition and nasal and laryngeal degeneration/hyperplasia supportable?*

Question 2): *A two-week range-finding inhalation study, evidently not available to the Hazard ID Committee, did not establish NOELs for cholinesterase inhibition or histopathology findings of nasal and laryngeal tissues at doses as low as 0.54 mg/L. Should this study influence the Hazard ID Committee decision not to evoke an uncertainty factor for acute risk assessment (i.e., 1-7 days) on the basis of cumulative effects*?

Question 3): *Should another study be required to identify the NOEL for the end points in question ?*

Question 4)*: Given the findings of nasal and laryngeal degeneration/hyperplasia in both of the recently submitted malathion and malaoxon combined chronic toxicity/carcinogenicity studies and the finding of rare nasal tumors in the malathion study, should the Agency require a carcinogenicity study by the inhalation route (e.g., inhalation exposure for first 90 days of a two year study)?*

Question 5): *Other than contributing to the completeness of the malathion data base, does this study provide any support for discounting a 10x safety factor imposed under FQPA for the protection of infants and children?*

V. Acute Neurotoxicity Study (Retinal Rosettes)

***Question 1**): Should retinal histopathology data be submitted for rats in the intermediate dose groups?*

***Question 2):** Should histopathology slides be submitted for independent examination by the Agency's pathologist (for anatomic features comparisons between control and treatment group lesions) as called for in the Data Evaluation Record (DER) for this study (a relatively simple request)?*

VI. Subchronic Neurotoxicity Study

***Question 1** Given the contrast between the NOEL of 1575 mg/kg/day (HDT) for female rats on neurotoxicity endpoints in this FIFRA Guideline study and that of the LOEL of 38 mg/kg/day (LDT) in the published work on a different set of neurotoxicity parameters, does the published work provide adequate reason or evidence to require a developmental neurotoxicity Guideline study, or another neurotoxicity study that embraces learning/memory , EEG, EMG, and possibly other neurotoxicity parameters not covered in the subchronic neurotoxicity Guideline study?*

***Question 2):** If the neurotoxicity findings in the published study are considered inadequate to trigger the additional Guideline testing, what criteria from published work, short of those upon which regulations could be directly based, might serve in that capacity?. (Note: Moeller and Rider (1962), a journal publication with attendant Guideline deficiencies, has served for decades as the basis for a regulatable end point (RfD) for malathion, while the publication in question here is only being put forth as sufficiently definitive to require a study in the FIFRA Guidelines heretofore not performed).*

VII Cholinesterase Inhibition - Enhanced Sensitivity of Females

***Question 1):** Does the malathion data base support a conclusion that females are the more sensitive gender with respect to cholinesterase inhibition by this organophosphate?*

***Question 2):** What approach might be taken to estimate, from the data currently available, a correction factor to be applied to the NOEL derived from the Moeller and Rider study in male human subjects to afford equivalent protection for women?.*

***Question 3):** Should additional testing in animal models be required to further quantitate the gender specific disparity?.*

ATTACHMENT 3: Conclusions of Ad hoc Neurotoxicity Subgroup

ISSUE #1--The possibly greater sensitivity of females (as compared to males) to the cholinesterase inhibiting effects of malathion, and how this sex difference might affect the RfD for this chemical.

Discussion: On November 6, 1997, the Hazard ID SARC decided to base the RfD for malathion

on the results of the 2-year combined chronic feeding/carcinogenicity study on rats (MRID 43942901). For the purpose of setting the RfD, the SARC considered the NOEL for inhibition of cholinesterase activity in this study to be 50 ppm in the diet (equivalent to 4 mg/kg/day in males and 5 mg/kg/day in females). A 32-56 day oral study in humans (males only)(Moeller and Rider, 1962) with a NOEL for inhibition of cholinesterase activity of 0.23 mg/kg/day was also discussed by the Hazard ID SARC and considered to be supportive of the RfD.

Subsequent to the November 6, 1978 meeting and during the neurotoxicity subgroup meeting on November 13, 1997, the issue was raised as to whether it would have been more appropriate to base the RfD for malathion on the results of the human study, rather than on the rat study. After considerable discussion, Clark Swentzel, in the capacity of chairman of the Hazard ID SARC, agreed to discuss this matter with selected members of the SARC to determine whether or not the full SARC might or might not be asked to readdress the choice of studies on which the RfD for malathion is based.

Regarding the possibly greater sensitivity of females (as compared to males) to the cholinesterase inhibiting effects of malathion, the results of cholinesterase determinations in numerous studies on malathion were discussed and it was agreed that females do indeed appear to be more sensitive than males. There was not full agreement, however, on the relative degree of increased sensitivity of females compared to males. Also, there was not full agreement on whether or not a modifying factor should be applied to the RfD for malathion if the human study (in which only males were tested) were eventually selected to be the study on which the RfD for malathion were based.

Recommendation: The consensus of the neurotoxicity subgroup was that if the human study were eventually chosen as the basis for the RfD, it would not be appropriate to apply an additional modifying factor to the RfD to account for the increased sensitivity of females as compared to males. The rationale for this recommendation was that although a sex difference in sensitivity apparently does exist, the difference appears to be small. In many (but not all) studies, the sex difference did not result in different cholinesterase NOELs for males and females, but rather in different degrees of cholinesterase inhibition for males and females at a given dose level. It was pointed out that NOELs, rather than degrees of effect at a given dose level, are used in HED to determine RfDs and as the basis for various other risk assessment calculations. It was also pointed out that this same issue (possibly greater sensitivity of one sex) had arisen several times in the past with respect to setting the RfD for other chemicals and that as a general policy it had previously been decided that additional modifying factors based on possible sex differences ordinarily would not be applied to RfDs.

The neurotoxicity subgroup also agreed that if the 2-year combined chronic feeding/carcinogenicity study in rats were retained by the Hazard ID SARC as the basis for the RfD, the question of whether or not to apply an additional modifying factor based on sex to the RfD would be "moot" since 50 ppm (equivalent to 4 mg/kg/day in males and 5 mg/kg/day in females) was the cholinesterase NOEL for both males and females in the study.

ISSUE #2--Should EPA require the registrant to submit the microscopic slides (or photomicrographs) of

retinal tissue from three rats in the acute and subchronic neurotoxicity studies on malathion?

Discussion: In the draft DER for the acute neurotoxicity study in rats (MRID 43146701), it was observed that 1/5 high dose group male rats had a bilateral retinal "rosette". Since concerns had arisen in recent years regarding the possibility that exposure to malathion might affect the visual system of humans and/or experimental animals, and since treatment-related lesions of the visual system had been observed in studies with certain other organophosphate pesticides, the occurrence of the bilateral retinal "rosette" in this high dose animal was considered by the reviewer to be a potentially serious effect of the test material and to warrant full investigation into the pathology and possible cause of the lesion in this animal. Further, the lesion was most likely a very rare event in rats. Toward this end, several pathologists were contacted regarding the potential seriousness of this lesion. These pathologists included Dr. Lucas Brennecke (EPA consulting pathologist), Dr. Robert Dahlgren (the study pathologist) and Dr. C. B. Clifford (Charles River pathologist). In addition, in the past, considerable discussion of this matter among several HED staff members also occurred, but all without resolution of the question of whether or not to ask the registrant to provide the microscopic slides of the retina of this rat to EPA for further examination--together with the slides of the retina of a control rat in the subchronic neurotoxicity study (MRID 43268501) which showed a unilateral retinal "rosette" and the slides of the retina from a randomly selected control rat from the acute study. Since the term "rosette" lacks histopathological preciseness, the slides of the retina of the control rat were required to determine if the lesion in this animal was indeed the same or was different than that in the high dose animal. Prior to the neurotoxicity subgroup meeting, additional information on retinal rosettes derived from a National Library of Medicine literature search was provided by Virginia Dobozy. The neurotoxicity subgroup discussed all the available information and data.

Recommendation: The consensus of the neurotoxicity subgroup was that, based on the presently available information, EPA should not ask for the microscopic slides of the retinas of these three rats at this time. The rationale for this recommendation included a weight-of the-evidence consideration of the following:

The lesion of concern (bilateral retinal rosette) occurred in only one high dose male rat in the acute neurotoxicity study.

A unilateral retinal rosette was also tentatively observed in one control male rat in the subchronic neurotoxicity study.

Drs. Brennecke and Dahlgren both concluded the retinal rosette in the high dose male rat was not of toxicological significance and was not due to treatment with malathion.

Dr. Dahlgren considered the cause to be a "developmental deficit which occurs at the time of retinal maturation".

The neurotoxicity subgroup also concluded that retinal rosettes in rats are most likely the result of abnormal proliferation and differentiation of developing retinal cells during neonatal life (i.e. during

the first approximately 32 days after birth) and ordinarily are not likely to develop in mature animals as a result of treatment with xenobiotics.

In a reference book available to the subgroup (Ophthalmic Pathology of Animals, Saunders and Rubin, 1975), it was stated that "[Retinal] rosettes occur spontaneously in certain strains of inbred rats and in beagle and collie dogs."

ISSUE #3--Should EPA require the registrant to perform and submit additional neurotoxicity studies to evaluate possible effects of malathion on learning and/or behavior and/or other neurological parameters as exemplified in a literature article by Desi et al. (1976) in which maze performance (learning) and EEG and EMG recordings were reported as being affected in rats treated with malathion?

Discussion: In the subchronic neurotoxicity study in rats (MRID 43269501), a guideline study that included a "functional observational battery" (FOB) and motor activity measurements, treatment-related effects on these two parameters were not observed at the highest dose level tested--20000 ppm (equivalent to 1486 mg/kg/day in males and 1575 mg/kg/day in females). However, in a non-guideline subchronic neurotoxicity study in female rats (reported by Desi et al., 1976), which employed dose levels of 0, 38 and 75 mg/kg/day, malathion was reported to affect maze performance (learning/memory) during the first 21 days of the study (increased errors and increased running time) and to affect EEG and EMG recordings after 90 days. At the dose levels tested in the Desi et al. study, brain cholinesterase activity was inhibited about 20% at 21 days, but clinical signs of cholinergic poisoning were not observed. Therefore, learning/memory deficits and changes in EEG and EMG recordings were reported in the absence of cholinergic clinical signs (i.e. at subclinical doses). Since the guideline subchronic neurotoxicity study (MRI(D 43269501) did not assess either learning/memory or EEG or EMG effects, it was recommended in the draft DER that the registrant be required to perform and submit additional neurotoxicity studies on malathion to evaluate possible effects on learning/behavior and EEG and EMG changes. A schedule-controlled operant behavior study (guideline 85-5) was suggested as a possibility. The neurotoxicity subgroup discussed the general subject of learning/behavior studies and also considered specific information pertinent to the Desi et al. study. In addition, a memorandum from R.C. MacPhail (Chief, neurobehavioral Toxicology Branch/HERL/EPA) to John Doherty (HED) and Brian Dementi (HED), dated May 4, 1995, was available which commented on the Desi et al. study and on the potential regulatory usefulness of further neurotoxicity testing of malathion as recommended in the draft DER.

Recommendation: The consensus of the neurotoxicity subgroup was that, based on the presently available information, EPA should not ask for additional neurotoxicity studies on malathion at this time. It was recognized, however, that such studies might possibly be requested at some time in the future if there were sufficient justification for doing so. Toward this end, the subgroup suggested it would be appropriate to perform a literature search on 1) learning/ behavior effects of organophosphates in general, and 2) available information on malathion in particular. After the literature search was completed and if warranted by new information, the question of additional neurotoxicity testing for malathion might be reconsidered.

ATTACHMENT 4: Dr. Dementi's Quotes from the External Peer Review Panel on the Acceptability of the Malathion Data Base (From Attachment 12 of February 28, 2000 Letter)

Dr. Dourson says "The lack of the monitoring of the critical effect in the developing offspring, and specifically, the lack of such measurement of RBC cholinesterase inhibition in the 2-generation study is a data gap....." (p. 3) "The specific question to be addressed with these data are whether or not the NOEL of the likely critical effect after 1 day exposure is determinable. The available data in this review, including the developmental studies in rabbits, do not allow this question to be answered." (p. 3) "No, the data on which to make this determination are absent." (p. 5) "However, I believe that the rat NOEL should be further divided by a 3-fold uncertainty factor to account for deficiencies in the data base...." (p. 8) "However, it does not test females, so the NOEL/LOEL range could be lower." (p. 8) His responses to both questions IV and V calling for additional information indicate his recognition of the existence of additional data gaps. A most significant statement made by Dr. Dourson reads as follows: *"I am not satisfied that the potential risk to humans is addressed with the data available in this review package." (p. 3)*

Dr. Hartung, beyond saying that a toxicology data base is never complete (p. 4), does not particularly address the question specifically for malathion. He does say the following: "The available data is inconclusive whether a single dose, administered during a day of maximum sensitivity would be able to elicit the observed response, or whether cumulative dosing is required." (p. 5) "This requires an analysis of the detailed cholinesterase methodology." (p. 7)

Dr. Decker: "The appearance of rarely-found malignant tumors in the nasal turbinates of 2 female rats should be a pointer that more animals should be tested to determine the incidence of said tumors in all dosage groups. The tumors should be further histologically defined." (p. 2) Along these same lines, he indicates that these findings "...demand further testing in a larger group of animals in all dosage groups." (p. 4) "The finding that the increased numbers of hepatocellular tumors observed in the male mice at 100 ppm as compared to the lower numbers of such tumors observed at 800 ppm is not interpretable, in my opinion. Rather, this part of the study should be repeated. The rest of the study seems to follow the Guidelines well, and appears to be scientifically valid." (p. 2) "I agree with the EXECUTIVE SUMMARY that this study is not acceptable and does not satisfy Guideline 83-1 for a chronic toxicity study in dogs because NOELs were not established for cholinesterase activity inhibition for plasma and erythrocytes in either sex." (p. 2) "Lacking an answer to this question, I would recommend that this DER be changed from CORE MINIMUM to UNACCEPTABLE for the section of the report on eye histopathology." (p. 3) "Although this study appears to satisfy the requirement of Guideline 82-7 for subchronic toxicity determinations, it was correctly pointed out in the Study Classification section that other published data indicate possible evidence of neurotoxicity on parameters not assessed in the 82-7 Guidelines. I recommend a thorough literature search on theses and that the results be used to construct additional specific neurotoxicity testing to assess for effects on learning, behavior, and EEG and EMG evaluations." (p. 3) "I agree with the Footnote on page 13 that the neurotoxicity and neurobehavioral testing g should be greatly expanded in scope, in light of development in these areas during the past decade. The DER should be put "on hold" until these changes are made." (p. 3) "This study seems to be generally acceptable, but does not satisfy all requirements of Guideline 82-4, since no NOEL was established for plasma and RBC cholinesterase inhibition in female animals or for microscopic lesions of the

nasal cavity of the larynx in both sexes." (p. 3) "I recommend that Dr. Dementi's suggestions be actively pursued, that is more studies are needed to fill in data gaps." (p. 4)

ATTACHMENT 5: Dr. Dementi's Consolidation of the Panel's Responses for Question 1, Issue 6, from Attachment 12

Comments: Dr. Dourson says no to this question for the reason that the LOEL of 38 mg/kg/day is not inconsistent with the cholinesterase NOEL in the 2-year rat study (a noteworthy observation in itself, attesting to the credibility of the non-Guideline study). He proposes applying a safety factor to the LOEL, which raises a concern analogous to that in the case of the inhalation study (Question IV), as to whether that is a suitable approach for these end points. The problems I find with this are: 1) the identification of an end point to be used for regulatory purposes, in this case the RfD based on cholinesterase inhibition, should be selected in light of what the collection of Guideline studies reveal, i.e. all Guideline testing requirements should be satisfied, ideally each having been pursued to the point of rational conclusion. Each type of study in the Guidelines has its purpose; 2) Behavioral effects are of the highest order of importance; 3) If indeed the findings in Desi et al should be corroborated to show that behavioral effects, effects on neurophysiological parameters (e.g. EEG, EMG) and cholinesterase inhibition occur in neurotoxicity studies at doses comparable to those of cholinesterase inhibition in the Guideline 2-year rat study, the RfD derived from the latter would then have enhanced meaning among those persons who argue that cholinesterease inhibition itself, in the absence of other effects, is of questionable concern; 4) The Desi et al study did not identify NOELs on the very important parameters mentioned, and more than speculation should be employed to say at what doses effects terminate; 5) Desi et al was conducted in the female rat, and a question remains whether the Guideline 2-year rat study identified a NOEL for erythrocyte cholinesterase inhibition in the female rat.

Dr. Hartung says, prior to answering this specific question: "The assessment needs to incorporate the entire harmonized data set from all studies. It should not depend upon a search for single values, which are then treated without context." (p. 3) He also says: "It would be desirable to have at least a brief discussion of the interrelations of the various cholinesterases at different sites, their functions, and their diagnostic utility in relation to OP poisoning." (p. 4) This is a tall order as we all know, and this is why the implications of studies such as Desi et al indicating correlations between cholinesterase inhibition and other effects at low doses should not be dismissed out of hand. I am puzzled by certain elements of his response to the question at hand. He says: "The studies in DER #10 and DER #11 show no behavioral effects at dose levels significantly above dose levels associated with plasma cholinesterase inhibition, but they do show abnormalities in EEG and EMG recordings after 90 days of exposure." (p. 10) Actually, in Desi et al (DER # 11) effects on the behavioral parameters were observed at both doses tested (38 and 75 mg/kg/day) as assessed at 21 days, at which time statistically significant cholinesterase inhibition (approximately 20%) of the cerebral cortex was observed at both doses as well as statistically significant erythrocyte cholinesterase inhibition (also approximately 20%) at the 75 mg/kg/day dose level. Dr. Hartung says: "The spread between simple behavioral responses and cholinesterase inhibition argues against a need for further study." (p. 10) The converse of this is that further testing <u>would</u> be indicated if the said spread were small, or non existant, as is true in this case. He indicates his uncertainty as to what end points could be evaluated in the developmental neurotoxicity study, and would thus want assurances as to its interpretability before proceeding. This suggests, but does not say, he would support such testing were the test(s) meaningful.

Dr. Hartung questions the reliability of Russian neurophysiology, but without some reference to that

literature with which to compare the work of Desi et al, it is difficult to appreciate any argument that the findings in Desi et al should not serve at least as a signal for definitive testing. It is documented in reliable sources that EEG is responsive to cholinergic agents, see Ref. U, and thus if EEG changes are noted in studies at doses close to, or particularly below, those that inhibit brain cholinesterase as assayed, this would be an important end point of probable regulatory concern.

Dr. Decker is firm in his recommendation that: "..... additional neurotoxicity testing to assess for effects on learning, behavior, and EEG and EMG evaluations."(p. 3), by the best methods available. He also says, with regard to DER #11: "I agree with the Footnote on page 13 that the neurotoxicity and neurobehavioral testing should be greatly expanded in scope, in light of developments in these areas during the past decade. The DER should be put 'on hold' until these changes are made." (p. 3)

In my view, the responses of Drs. Hartung and Decker support a requirement for additional neurotoxicity testing that would be designed to reconcile the contrasting findings between the published and Guideline subchronic neurotoxicity studies in question. It is important to mention here as discussed elsewhere in this document that the publication by Kurtz (1977) (Ref. D) reveals a behavioral response to malathion within (actually below) the dose range that inhibited cholinesterase. The Guideline developmental neurotoxicity study, with some add-on testing, might be suitable to address the issue. While Dr. Dourson responds in the negative, his rationale does not incorporate or indicate consideration of the important issues being raised pertaining to neurotoxicity testing.